Vol. 28 No. 2

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Editor

HAMILTON B. G. ROBINSON

APRIL, 1949

The Editor's Viewpoint ---------- 95

Pulp Healing. R. L. Glass and H. A. Zander (Tufts College Dental School, Boston, Mass.) ---------- 97

Maintenance of Equilibrium in Reversible Hydrocolloid Impressions. I. Made With Stock Impression Materials. Allison G. James (Beverly Hills, Calif.) ---------- 108

Maintenance of Equilibrium in Reversible Hydrocolloid Impressions. II. Made With Materials Supplied by the Manufacturers for Testing. Allison G. James (Beverly Hills, Calif.) ---------- 119

The Effect of Wetting Agents on Oral Lactobacilli. II. Local and Systemic Toxicity in Mice. Toxicity vs. Antibacterial Activity. Frank E. Walter and Harve J. Carlson (School of Medicine, Western Reserve University, Cleveland, Ohio) ---------- 124

(*Contents continued on inside back cover*)

Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
By The C. V. Mosby Company, St. Louis 3, Mo.

Copyright 1949 by The International Association for Dental Research

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee—J. Frank Hall, S. S. Arnim, and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson (Ohio State University). *Business Manager*—J. Frank Hall (Indiana University). *Circulation Manager*—S. S. Arnim (University of Texas).

Assistant to the editor—Paul C. Kitchin and J. B. Brown. *Assistant to the business manager*—Henry M. Swenson. *Assistant to the circulation manager*—J. William Adams.

Board of Editors—Editor-emeritus, members of the Publication Committee, and sectional representatives named in fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1948)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
Canada	Halifax	S. G. Ritchie	J. S. Bagnall	S. G. Ritchie
	Toronto	A. W. Ellis	A. D. Mason	Thos. Cowling
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
China	Chengtu	R. Gordon Agnew	R. Gordon Agnew	R. Gordon Agnew
England	London	Evelyn Sprawson	Evelyn Sprawson	Evelyn Sprawson
Czechoslovakia	Prague	Friedr. Neumann	Friedr. Neumann	Jan Jesensky
Austria	Vienna	Fritz Driak	R. Trauner	F. Shönbauer
Hungary	Budapest	Ferenc Köszeg		
Palestine	Palestine	S. Lewin-Epstein	S. Lewin-Epstein	S. Lewin-Epstein
South Africa	Johannesburg	Julius Staz	J. N. Noriskin	W. B. Osborn
United States	Ann Arbor	Philip Jay	Philip Jay	Philip Jay
	Baltimore	M. S. Aisenberg	E. C. Dobbs	J. B. Robinson
	Boston	R. F. Sognnaes	J. F. Volker	C. M. Waldo
	Chicago	J. P. Weinmann	S. F. Bradel	G. B. Denton
	Cleveland	S. W. Chase	Thomas J. Hill	S. W. Chase
	Columbus	H. B. G. Robinson	L. F. Edwards	P. C. Kitchin
	Detroit	Samuel J. Lewis	P. C. Lowery	Samuel J. Lewis
	Houston	F. C. Elliot	F. C. Elliot	F. A. Peyton
	Indianapolis	Henry M. Swenson	Maynard K. Hine	R. W. Phillips
	Iowa City	A. O. Klaffenbach	A. W. Bryan	George S. Easton
	Louisville	Raymond E. Myers	D. Y. Burrill	D. Y. Burrill
	Minnesota	W. J. Simon	W. M. Crawford	A. P. Lund
	New Orleans	T. D. Speidel	H. W. Peterson	F. J. Wolfe
	New York	Isaac Neuwirth	S. C. Miller	F. Krasnow
	Pacific Northwest	N. M. Phatek	H. J. Noyes	B. O. A. Thomas
	Philadelphia	Ned Williams	Paul Boyle	S. L. Rosenthal
	Pittsburgh	L. E. Van Kirk	H. E. Friesell	
	Richmond	Herbert D. Coy	Alton D. Brashear	Wendell O. Clough
	Rochester	K. L. Schourie	D. B. Mitchell	N. S. Simmons
	San Francisco	S. Epstein	W. C. Fleming	Herman Becks
	St. Louis	W. B. Gurley	R. F. Bleiker	L. R. Boling
	Tennessee		O. A. Oliver	M. T. Dean
	Washington	H. J. Caul	F. S. Arnold	B. E. Erickson
Materials Group		F. A. Peyton	E. W. Skinner	N. O. Taylor

OFFICERS OF THE ASSOCIATION

President—A. G. Brodie, *President-elect*—J. R. Blayney, *Vice-president*—B. G. Bibby, *Secretary-Treasurer*—Edward H. Hatton, *Editor*—Hamilton B. G. Robinson, *Trustees*—R. W. Bunting, Edward H. Hatton, Thomas J. Hill, Leuman M. Waugh, J. L. T. Appleton.

Vol. 28, No. 2, April, 1949. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $6.00 per volume; Canada and other foreign countries, $6.50. Single number when available, $1.25. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the act of March 3, 1879. Printed in the U. S. A.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Nov. 10, 1946), 627. *Eligibility (Const., Art. 14, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of Mar. 16, 1946): $1,402.98. There have been no expenditures from this fund.

INFORMATION FOR PROSPECTIVE CONTRIBUTORS, SUBSCRIBERS, OR ADVERTISERS

The JOURNAL OF DENTAL RESEARCH was dedicated by its founder to the dissemination of knowledge pertaining to the teeth and mouth, and to their relations to the body as a whole. It publishes original investigations in these and related fields. Articles are accepted only on condition they are contributed solely to this JOURNAL. Articles appearing in other domestic or foreign periodicals will not be reprinted in the JOURNAL except in extraordinary cases. The JOURNAL will reproduce a limited number of half-tones or zinc etchings without cost to the author. References to the literature must conform to the following style:

1. (To a periodical—Doe, J. A.: Dental Disease, *J. D. Res.* **16**: 42, 1937.
2. (To a book)—Doe, J. A.: The Dental Practice, ed. 2, Chicago, 1930, Smith, ch. 2, p. 114.

See McBride, *J. D. Res.* **16**: 537, 1937.

Titles should be given for all references. Papers presented at society meetings should be so footnoted. Reprints, up to 500 copies, will be supplied at prices quoted by the publisher after the JOURNAL is in the mail, and must be ordered from the publisher. Additional reprints may be purchased only at the discretion of the Board of Editors.

Subscription price for the volume (issued bimonthly beginning with February) is $6.00. Single copies $1.25. The price of volumes and single numbers prior to 1948 will be quoted upon request to the office of the Business Manager, 1121 W. Michigan St., Indianapolis, Ind.

Matters pertaining to subscriptions, change of address, and other business matters should be addressed to The C. V. Mosby Company, Publishers, 3207 Washington Blvd., St. Louis 3, Mo.

Address all communications relating to editorial matters to the JOURNAL OF DENTAL RESEARCH, Ohio State University, College of Dentistry, Columbus 10, Ohio; advertising or business matters to the JOURNAL OF DENTAL RESEARCH, The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo.

THE EDITOR'S VIEWPOINT

The term "biologic" is often used in discussing the technics of dentistry, dental education, and the practice of dentistry. Often biologic relationships are rather tenuous, but Glass and Zander (p. 97) discuss one of the important biologic applications in root canal therapy in the treatment of diseased pulps. The ability of the pulp to heal has not been generally recognized, but careful investigations have shown that under proper conditions sound young pulps will show healing which is characterized by repair and the formation of a new dentin barrier. Testing two materials commonly used in the treatment of exposed pulps, zinc oxide, eugenol and calcium hydroxide water paste, the authors have demonstrated that while the pulp may remain vital under zinc oxide eugenol treatment, a chronic inflammatory reaction persists at the site of exposure and repair does not take place. They were able to show that when exposed pulps are capped with calcium hydroxide, a rapid healing process is seen with relatively little inflammatory reaction. Within four weeks, the site of exposure was completely walled off by a new odontoblastic layer and a new dentin barrier. Contributions such as these have immediate clinical application and the biologic implication is sound and clear cut. The clinician who encounters exposed sound young pulps certainly should use calcium hydroxide water paste. Laboratory studies, such as that reported by Glass and Zander, and clinical studies have shown the value of such therapy.

James (p. 108) tested the accuracy of impressions made from reversible hydrocolloid impression materials after immersion for periods of time in water or potassium sulfate solution as commonly recommended. He found that one commercially available material expanded and another contracted within a critical time. The results of these tests indicate that impressions made in hydrocolloid materials should be poured immediately. The change with time may be the reason for failure of this material in the hands of some individuals and marked success in others.

James (p. 119), making further studies of materials supplied by manufacturers, still found that storage of hydrocolloids reduced accuracy. Findings with these fresh materials indicate that shelf storage may be a factor. Water was better for storage than was 2 per cent potassium sulfate solution. The author appears justified in his suggestion that manufacturers should not infer that storage in water or potassium sulfate solution is a safe procedure.

A great number of wetting agents have been developed in the past few years. These surface-active agents are in many instances valuable anti-bacterial substances. Walter and Carlson (p. 124) investigated the toxicity of 69 such agents. They found that, in general, cationic agents were highly toxic in the technic used; the nonionic were less toxic; and the anionic agents were in general, nontoxic, although some produced pathological lesions. The effectiveness of these various agents against bacteria was investigated. Some of the anionic agents of low toxicity were quite effective against gram-negative organisms. This basic work is useful in evaluating the possible effectiveness of surface-active agents against infectious oral diseases, including dental caries.

Keyes and Shourie (p. 138) gave fluorides to hamsters as sodium fluoride in food, in water, and subcutaneously, as calcium fluoride in food, and as sodium fluorosilicate in food. The sodium fluoride effectively reduced caries activity whether given in food or in water, sodium fluorosilicate was less effective, and calcium fluoride "essentially ineffective." Because of contamination of the mouth by urine and feces, the subcutaneous injection study was not considered as conclusive. The availability of the F ion in the oral cavity appears to be highly important in posteruptive caries control. This fact should be carefully considered by those who advocate the use of relatively stable fluoride compounds as a means of control for caries.

Irving (p. 144) experimentally has demonstrated that the blood calcium level causes premature calcification of the experimental dentin line produced in rats by injecting sodium fluoride. The relationship between calcification, calcium, and fluorides is of importance not only in experimental studies of dentin and enamel formation, but it may offer a clue to unsolved problems in dental fluorosis and in caries suppression by fluorides.

Zipkin and McClure (p. 151) used littermate rats to study the effect of fluorides in controlling tooth substance loss by citrate and lactate ions. It was found that 50 ppm. of fluorine afforded no more protection against the type of erosion produced by citrate at pH 6.3 to 6.5, than did 5 ppm. of fluorine, but that increasing protection was given in the order of 1, 2.5, and 5 parts per million. Against erosion by lactate (at pH 4.5), 2.5 ppm. of fluoride was most effective. The authors believe that a minimum concentration of fluorine exists, and beyond this further increases in fluorine give no additional protection against these types of erosion in rats. Fluorine, acquired by intraperitoneal injection was capable of reducing erosion produced by citrate but not that produced by lactate. However, even against citrate, comparatively large doses were required when given intraperitoneally. How these studies of the action of fluorides on this peculiar type of dental destruction in the rat apply to human tooth disease has not been determined.

Manly and Bibby (p. 160) tested the efficiency of 147 substances (compounds of 57 elements), in reducing the acid solubility of powdered enamel in vitro. They found twenty-seven of these compounds to be more effective than 0.1 per cent aqueous solution of sodium fluoride in reducing enamel solubility under the conditions of the study. It should be noted, however, that after treatment in lead fluoroborate and acid, the enamel was actually heavier than before treatment. Whether adsorption of other heavy materials had a similar effect should be considered. The authors discuss some of the possible reasons for discrepancies between enamel solubility studies and clinical results but appear to have overlooked the possibility that reduction in enamel solubility may play only a part, whether a large or a small one, in the process of caries control by such compounds as sodium fluoride.

English (p. 172) reports a study of the effects of two goitrogenic compounds, thiouracil and selenium, on the teeth and jaws of dogs. It is interesting that hypoplasia of enamel and other signs of disturbance of metabolism occurred. The author notes the similarity between the changes observed and those produced by dietary deficiencies. This is not surprising since the dietary deficiencies and endocrine disturbances are both disturbances in metabolism producing the same type of pathologic lesion, just as disturbances in growth, genetic disturbances, trauma, and invasion by microorganism result in tissue changes following general patterns. It is unfortunate that mongrel dogs, with only one control, were used. As Dr. English notes, variations within a litter of mongrel dogs are often great.

Erausquin (p. 195) has made a detailed study of the bands of Schreger and presents an explanation for their concentric appearance in horizontal sections. The schematic drawings are quite lucid.

H. B. G. R.

PULP HEALING

R. L. GLASS AND H. A. ZANDER

Tufts College Dental School, Boston, Massachusetts

THE treatment of exposed pulp remains a controversial subject because no criteria for pulp healing have been established. Pulp capping is performed to maintain the vitality of a tooth; it is justifiable only if healing results under the capping material. Most pulp-capping experiments in the past have been evaluated on a clinical or roentgenological basis only. Such investigations appear to be concerned with the lack of adverse symptoms rather than the actual healing process. However, there exists no clinical method by which one can determine whether or not healing results. It is a well-known fact that pulp degeneration and necrosis may occur without any clinical signs or symptoms. A roentgenogram shows only those gross pathological changes which may or may not result at the apex of a tooth. Furthermore, vitality tests may indicate a vital tooth although the entire coronal portion of the tooth may be necrotic. Therefore, clinical studies using these methods of evaluation give only hazy results that should be substantiated by histological study.

In general, healing may be defined as the restoration of a tissue to its normal structure and function. The dental pulp is normally encapsulated by dentin and an adherent odontoblastic layer. Therefore, in applying this definition to the pulp, healing means the presence of healthy tissue capable of carrying on the function of the pulp, a continuous odontoblastic layer, and the erection of a dentin barrier walling off the exposure.

The object of this investigation was to determine whether or not, and under what conditions an exposed, healthy, young pulp is capable of healing according to the above definitions.

REVIEW OF THE LITERATURE

Dentists for a long time have believed the pulp to be capable of healing. The first mention of pulp capping is found in 1756 when Philip Pfaff[1] attempted to cap exposed pulps with a small piece of gold carefully adapted to the base of the cavity. In 1826 Leonard Koeker[2] cauterized the exposed portion of the pulp with a red hot iron wire, then covered the exposure with a piece of lead foil. Since this date there have been virtually as many technics of pulp capping as dentists. The earliest attempts to promote pulp healing consisted of placing different metallic foils against the site of the exposure. This is not illogical, since certain metallic splints are used effectively in the treatment of bone fractures today. The use of drugs and other materials in close contact with the

This investigation was supported by a Research Grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

Presented at the 26th general meeting of the International Association for Dental Research, Rochester, N. Y., June 18-20, 1948 (*J. D. Res.* 27: 744, 1948).

Received for publication July 9, 1948.

pulp gradually came into widespread use. Among the materials recommended to promote healing of accidental and pathological exposures are the following: asbestos, plaster of Paris, powdered ivory, tissue paper (and) Canada balsam, aseptic sponge, vulcanized rubber, court plaster, gutta percha, cork, quill, oiled silk, gall nuts, beeswax, pulverized glass, zinc oxychloride cements, collodion, boracic acid, iodoform powder, iodoformagen, formaldehyde, lactophosphate of lime, borax, zinc oxide, zinc oxide eugenol, zinc oxide and thymol, $Ca3(PO_4)_2$ and eugenol, $CaCO_3$, CaF_2, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$ and eugenol, $Ca(OH)_2$ and eugenol, $MgPO_4$, CaF_2, and water, and zinc phosphate cements.

In addition to these materials listed, there is a great variety of commercial preparations with known and unknown formulas. For each of the above drugs, there is ardent testimony as to ease of manipulation and successful clinical results. However, there is little or no controlled experimental evidence to substantiate their use to promote pulp healing. Five to ten years later such articles are followed by refutations and recommendations for new materials and technics. One would gather that a dentist finally saw many of his pulp cappings return to his office as aching and/or nonvital teeth.

White in 1859[3] and Grove as late as 1928[4] believed the dental pulp, like other specialized tissues, to be incapable of regeneration; thus they discarded attempts at pulp capping as impractical. Their ideas were substantiated in part in 1927 by Palazzi,[5] who exposed aseptically the pulps of dog teeth without causing any hemorrhage. These exposures were capped with asbestos and metal discs not in contact with the pulp. Palazzi found that the pulp underwent degeneration and failed to heal. In contrast are the histological studies of pulp amputations[6, 7] which show that the pulp can heal. There is definite histological evidence that a bridge of dentin is formed at the line of amputation. Such a bridge could not be formed unless the dental pulp is capable of healing.

EXPERIMENTAL PROCEDURE

To determine whether a sound, young pulp that is deliberately exposed is capable of healing, the following experiment was carried out. Without anesthesia, occlusal or gingival third cavities were prepared under a warm water spray in sound bicuspid teeth to be extracted for orthodontic purposes. After a classical cavity preparation was made, the pulp was exposed. The tooth was then isolated with cotton rolls, the cavity dried with compressed air, and the exposure was capped with one of the following materials: (1) a paste of zinc oxide and eugenol or (2) a paste of calcium hydroxide and tap water. The remainder of the cavity in either case was filled with zinc oxide and eugenol. Only patients losing a minimum of two teeth were included in this study, so that healing could be studied under different conditions in the same patient. Zinc oxide eugenol and calcium hydroxide water pastes were used as capping materials under which to study healing, because of the acceptance of the former as a bland filling material and the successful results with the latter in amputation experiments. A total of 40 sound teeth from children 9 to 15 years of age were used.

These teeth were extracted at intervals of 24 hours, 2, 4, 6, 8, and 12 weeks after the pulp capping was performed. The extracted teeth were immediately ground into longitudinal slabs, fixed in Zenker formol, decalcified in 5 per cent nitric acid, and prepared in the usual manner for celloidin sections, which were stained with hematoxylin and eosin.

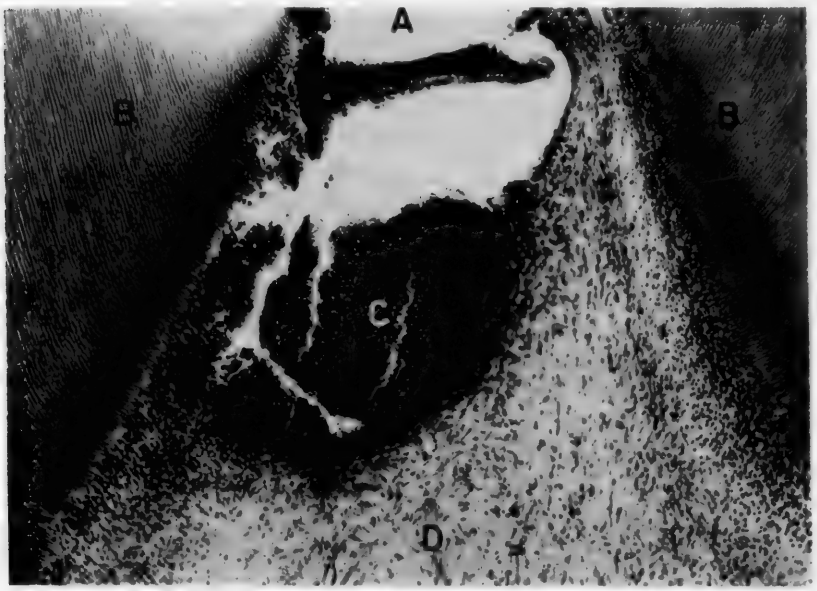


Fig. 1.—Exposure 24 hours after capping with zinc oxide eugenol. *A*, site of exposure, *B*, dentin walls, *C*, hemorrhagic mass, and *D*, pulp.

RESULTS

A marked difference in healing capacity is observed between exposed pulps capped with zinc oxide eugenol and calcium hydroxide. An exposure 24 hours after it was capped with zinc oxide eugenol paste shows below the site of the exposure a mass of red blood cells and polymorphonuclear leukocytes (Fig. 1). The hemorrhagic mass is demarcated from the underlying pulp tissue by a zone of fibrin and inflammatory cells. There is hemorrhage into the coronal portion of the pulp and a moderate influx of polymorphonuclear leukocytes. In sharp contrast is a 24-hour exposure capped with calcium hydroxide (Fig. 2). There is no hemorrhagic mass and but few inflammatory cells. However, the superficial portion of the pulp that was in contact with the calcium hydroxide is necrotic; there is little or no cellular detail present. This necrotic area is demarcated from healthy pulp tissue below by a new, deeply staining zone, a high power view of which is seen in Fig. 3. This zone is comprised of basophilic elements that are probably made up of a calcium proteinate. Calcium hydroxide being basic, with a pH around 11, necrotizes the superficial tissue;

the location of the proteinate globules probably represents the depth of penetration of the calcium hydroxide, which finally reacts with tissue protein to form this new structure.

Two weeks after capping with zinc oxide eugenol, degeneration is apparent at the site of the exposure and a chronic inflammation exists in the pulp tissue below (Fig. 4). Lymphocytes, plasma cells, and polymorphonuclear leukocytes are seen around the site of the wound; there is hyperemia and hemorrhage in the underlying tissue. A two-week-old exposure from the same patient, but capped with calcium hydroxide, shows a healing process that is well under way (Fig. 5). A portion of the superficial pulp tissue necrotized by the calcium hydroxide remains at the site of the exposure. The original proteinate zone of demarcation seen in a 24-hour calcium hydroxide specimen is still present; against this zone there is a new area of coarse fibrous tissue, shown in higher magnification in Fig. 6. This fibrillar structure may be likened to the fibrous or primitive type bone found in rapid bone healing. Like primitive bone, this fibrillar structure or primitive dentin is probably only partially calcified, since calcification tends to lag behind matrix formation. On the periphery of this new fibrous tissue, cells resembling odontoblasts appear to be lining up. This is the earliest sign of differentiation of new odontoblasts. The pulp tissue below is normal and is free of inflammation.

An exposure four weeks after capping with zinc oxide eugenol shows at the site of the exposure an increasingly larger zone of chronic inflammation (Fig. 7). Polymorphonuclear leukocytes, lymphocytes, and plasma cells are present. The dentin fragments forced into the pulp are undergoing resorption in some places; new dentin is being deposited around other areas to join these fragments together. An exposure from the same patient extracted four weeks after capping with calcium hydroxide is shown in Fig. 8. The superficial tissue necrotized by the calcium hydroxide has fallen out. The proteinate zone of demarcation seen in the original 24-hour specimen is still visible. Against the fibrillar structure formed after two weeks, a well-defined zone of new dentin has been deposited with an adherent layer of new odontoblasts—both continuous with the walls of pulp chamber. Fig. 9, a high magnification of Fig. 8, shows in detail the newly formed dentin and odontoblasts. The tissue below is normal. The pulp has healed.

Even after eight weeks an exposure capped with zinc oxide eugenol has not healed (Fig. 10). The original site of the exposure is walled off only by chronic inflammatory cells. The coronal pulp tissue below is mildly infiltrated by lymphocytes and polymorphonuclear leukocytes. The remainder of the pulp is essentially normal. New dentin is being deposited around the dentin fragments forced into the pulp. An exposure from the same patient, capped for eight weeks with calcium hydroxide, shows an advanced stage in the healing process (Fig. 11). The same elements seen in a four-week specimen are present; however, the dentin barrier is thicker and the odontoblasts appear to be more regularly aligned.

Fig. 2.—Exposure 24 hours after capping with Ca $(OH)_2$. *A*, site of exposure, *B*, dentin walls, *C*, necrotic area, *D*, line of demarcation and calcium proteinate precipitation, and *E*, pulp.

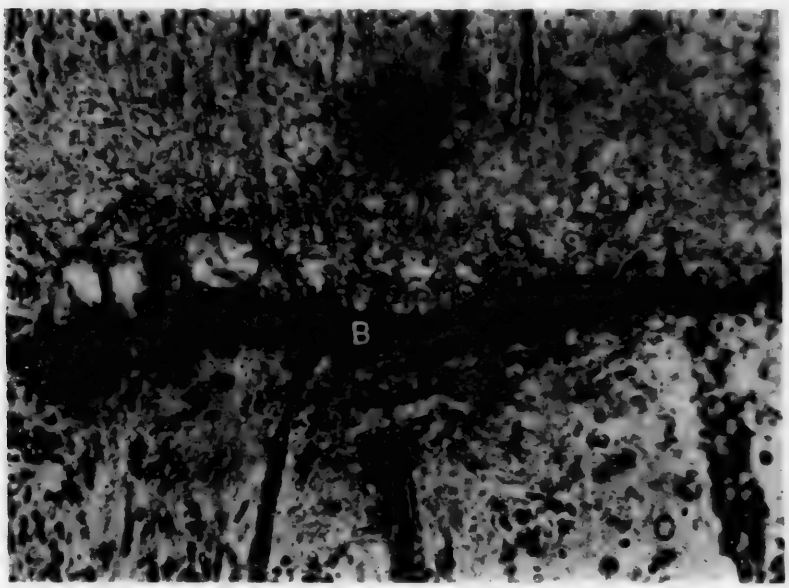


Fig. 3.—High magnification of line of demarcation shown in Fig. 2. *A*, necrotic area, *B*, line of demarcation and calcium proteinate precipitation, and *C*, vital pulp tissue.

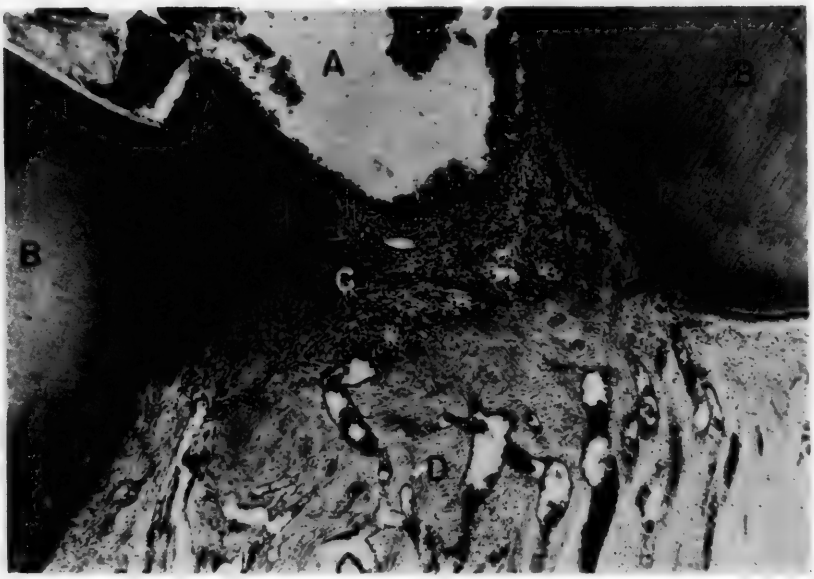


Fig. 4.—Exposure two weeks after capping with zinc oxide eugenol. *A*, site of exposure, *B*, dentin walls, *C*, area of chronic inflammation, and *D*, area of hyperemia and hemorrhage.

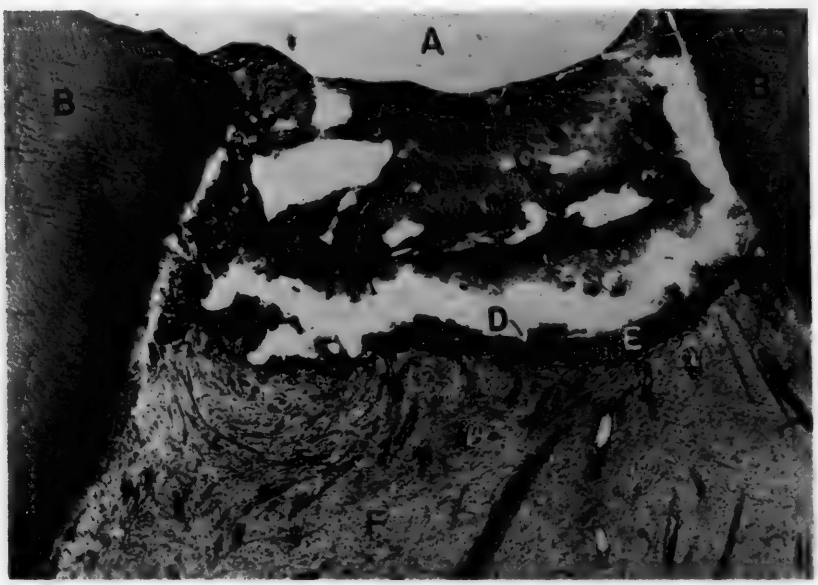


Fig. 5.—Exposure two weeks after capping with Ca $(OH)_2$. *A*, site of exposure, *B*, dentin walls, *C*, necrotic area, *D*, line of demarcation, *E*, area of fibrous proliferation, and *F*, vital pulp.

Fig. 6.—High magnification of areas *D* and *F* in Fig. 5. *A*. line of demarcation, *B*. fibrous proliferation, *C*. capillary enclosed by fibrous proliferation, *D*. alignment of pulp cells which are to differentiate into odontoblasts, *E*. nerve, and *F*. capillary.

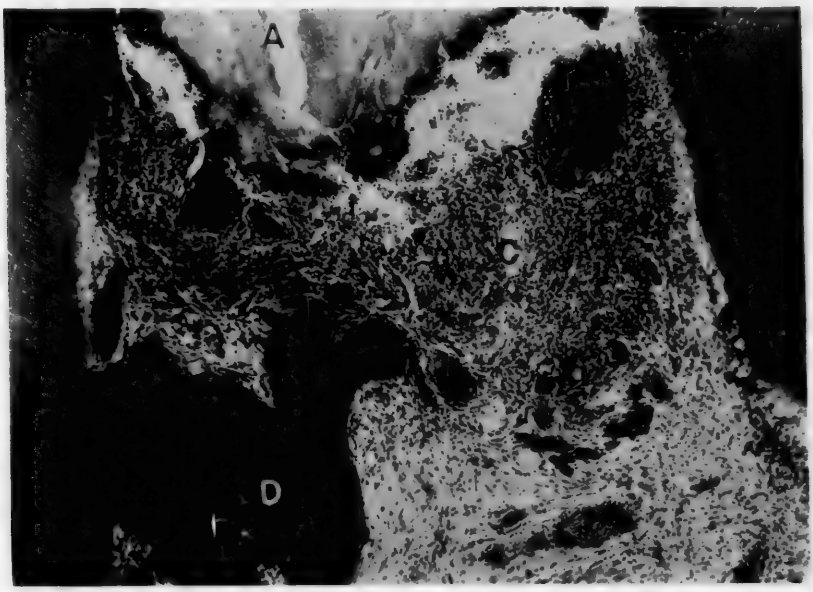


Fig. 7.—Exposure four weeks after capping with zinc oxide eugenol. *A*. site of exposure, *B*. dentin walls, *C*. area of chronic inflammation, and *D*. dentin fragments.

Fig. 8.—Exposure four weeks after capping with Ca $(OH)_2$. *A.* site of exposure, *B.* dentin walls, *C.* remnant of necrotic area, *D.* zone of demarcation and primitive dentin, and *E.* new dentin barrier.

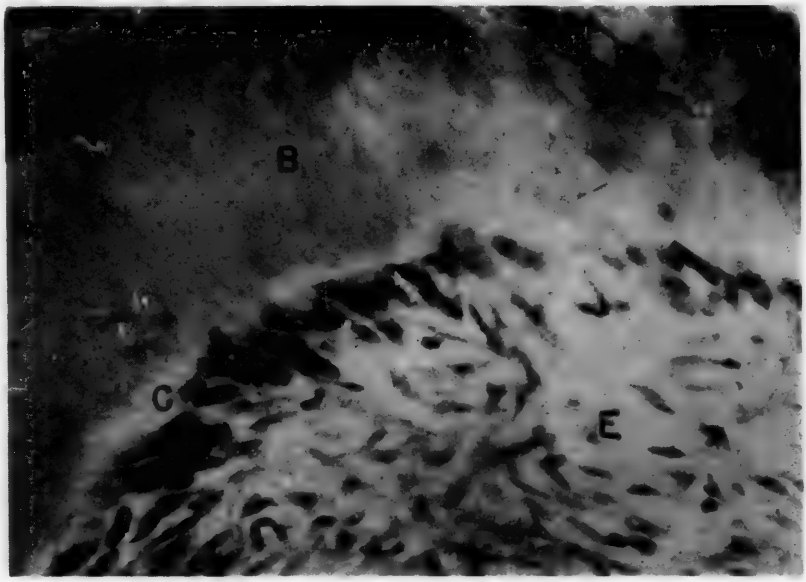


Fig. 9.—High magnification of areas *D* and *E* in Fig. 8. *A.* primitive dentin, *B.* new dentin barrier, *C.* predentin, *D.* odontoblasts, and *E.* pulp.

DISCUSSION

Originally, healing of an exposed pulp was defined as the walling off of the exposure by new dentin formation. According to this dictum, sound, young pulps capped with calcium hydroxide are healed within four weeks (Fig. 8). Zinc oxide eugenol failed to promote healing of exposed, sound, young pulps. Although pulps capped with zinc oxide eugenol remained vital and without clinical symptoms during the course of this experiment, a chronic inflammatory reaction persisted at the site of the exposure. This demonstrates the inadequacy

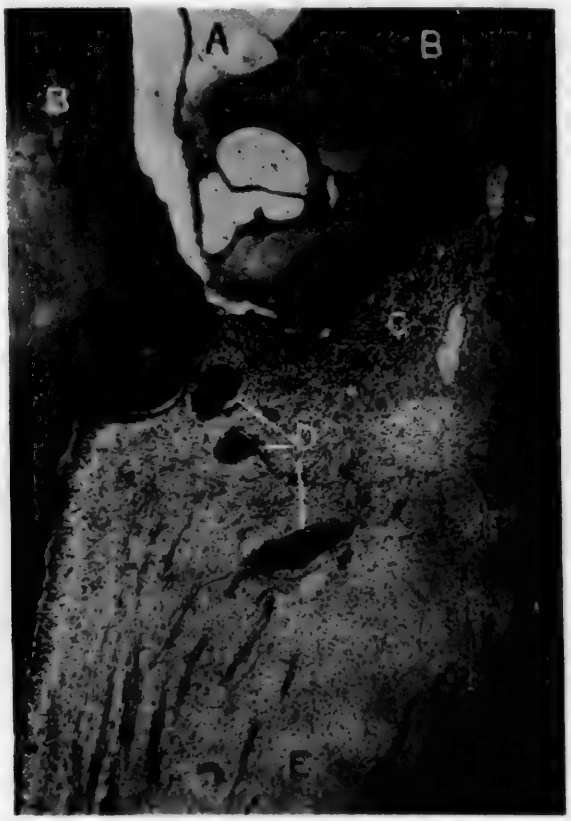


Fig. 10.—Exposure eight weeks after capping with zinc oxide eugenol. *A*, site of exposure, *B*, dentin walls, *C*, area of chronic inflammation, *D*, dentin fragments, and *E*, normal pulp.

of clinical evaluation alone of such experiments. In several cases, new dentin was deposited around dentin fragments forced into the pulp. In one case new dentin was deposited on the wall of the pulp chamber in an attempt to constrict the diameter of the pulp and join dentin fragments to form a barrier. The only sign of healing observed was this attempt of the pulp to join dentin fragments forced into the pulp by a burr at the time of exposure. In no case was an exposure capped with zinc oxide eugenol completely walled off by new dentin formation during the 12-week duration of this study.

On the other hand, the previously listed criteria for pulp healing are seen in the healing of exposures treated with calcium hydroxide. Within four weeks, the continuity of the odontoblastic layer has been restored, the exposure has been walled off by new dentin formation, and the pulp is normal. When calcium hydroxide comes in contact with exposed pulp tissue, there occurs within 24 hours a linear deposition of a calcium proteinate, forming a basophilic zone that sharply demarcates normal pulp tissue from the superficial area necrotized by the basic calcium hydroxide. Against the proteinate zone there occurs a proliferation of coarse, fibrous elements that undergo calcification to form what may be called a "primitive" type of dentin. After two weeks new odontoblasts start to differentiate and a barrier of new dentin is formed to join the walls of the pulp chamber to complete the healing process.

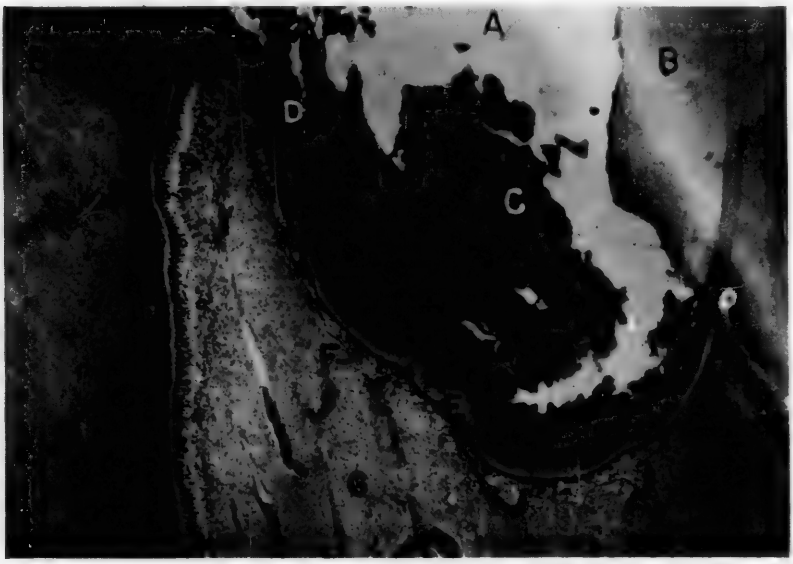


Fig. 11.—Exposure eight weeks after capping with Ca $(OH)_2$. *A*, site of exposure, *B*, dentin walls, *C*, remnant of necrotic area, *D*, zone of demarcation and primitive dentin, *E*, new dentin barrier, *F*, continuous odontoblastic layer, and *G*, vital pulp.

SUMMARY

Pulp healing under two commonly used materials, zinc oxide eugenol and calcium hydroxide water pastes, has been studied in exposed, sound young pulps. No healing was observed in exposed pulps capped with zinc oxide eugenol; pulps so treated remained vital, but a chronic inflammatory reaction persisted at the site of the exposure. However, when exposed pulps are capped with calcium hydroxide a rapid healing process is seen, relatively free of inflammation. Within four weeks the original site of the exposure is completely walled off by a new odontoblastic layer and a new dentin barrier.

REFERENCES

1. Pfaff, P.: Abhandlung von den Zaehnen des menschlichen Koerpers und deren Krankheiten, Berlin, 1756.
2. Koeker, L.: Principles of Dental Surgery, London, 1826, Underwood, p. 433-437.
3. White, J. D.: Practical Hints, *D. Cosmos* **1**: 618, 1860.
4. Grove, C. J.: Capping Vital Pulps, *J.A.D.A.* **15**: 1688, 1928.
5. Palazzi, S.: Experimentelle Untersuchungen uber das Problem der Heilungsvorgange in de blosstgelegten gesunden Pulpa, *Ztschr. f. Stomatol.* **25**: 91, 1927.
6. Teuscher, G. W., and Zander, H. A.: A Preliminary Report on Pulpotomy, *Northwestern Univ. Bull.* **39**: 4, 1938.
7. Zander, H. A.: Reaction of the Pulp to Calcium Hydroxide, *J. D. Res.* **18**: 373, 1939.

MAINTENANCE OF EQUILIBRIUM IN REVERSIBLE HYDROCOLLOID IMPRESSIONS

I. Made With Stock Impression Materials

ALLISON G. JAMES, D.D.S.

Beverly Hills, Calif.

INTRODUCTION

WHAT happens to reversible hydrocolloid impressions in the interval between making the impression and pouring the model is important. There is general agreement that the shorter this interval is the greater the accuracy obtained; nevertheless, many dentists send impressions to commercial laboratories for pouring and a time lapse of hours to days may ensue. This may affect seriously the accuracy of the resultant model. Some manufacturers state: "The impression may be preserved by wrapping in wet cloth or paper"; others: "by immersing in a dilute solution of potassium sulphate (about 2%)." Laboratories receiving partially dehydrated impressions usually place them either in water or the potassium sulfate solution, frequently of unknown strength, prior to pouring the models. It has been thought that the preservation of osmotic balance was adequate to check both synaeresis (the property of expelling water by contraction) and rehydrating or imbibing water with possible rupture. This conclusion apparently was reached without adequate tests. The matter now appears more complex than this.

Up to this time standards for reversible hydrocolloid impression materials have been based on partial denture uses.[2] Inlays and fixed bridges require greater accuracy than is commonly considered acceptable for partial denture impressions. The impressions not only must be accurate, but they must be maintained in perfect equilibrium until the models have set if gross inaccuracies are to be avoided. The following tests were devised to learn the maximum time interval that accuracy could be maintained between making an impression and pouring the model.

MATERIALS AND METHODS

Part 1

In testing materials for experimental models, extracted natural teeth and casein teeth were found to absorb water and change volume under repeated immersions in making impressions. Therefore porcelain tube teeth were selected, although they do not permit making sharp line angles as preparations must be made with revolving stone instruments. They are, however, hard, strong, nonporous, and the thermal expansion negligible in the temperature range used. Three identical maxillary first molar porcelain tube teeth were prepared as follows:

1. *Mesio-occlusal cavity.* In this type the inlay casting will be entirely contained within the preparation.

Received for publication June 14, 1948.

2. *A three-quarter crown.* In this type the casting will be almost entirely outside and surrounding the preparation.

3. *Mesio-occluso-distal with a distolingual veneer.* In this type the casting will be a combination of within and surrounding the preparation.

These preparations were mounted in artificial stone in display rings. Specially constructed impression trays were made and a wood block, weighted to prevent floating and capsizing while chilling the impression, devised which would insure a similar bulk of impression material for each impression made (Fig. 1).

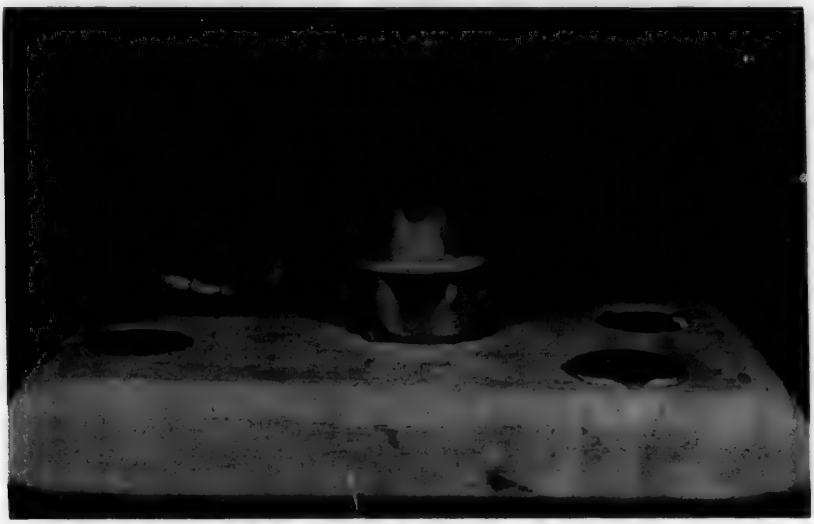


Fig. 1.—Impression tray, block, and porcelain preparation. Block insures similar amount of impression material for each impression made and is weighted to prevent floating and capsizing during chilling.

This paper describes the tests of two materials. Table I gives the materials tested, identified by their code letters, together with the manufacturer's name.

TABLE I

MATERIALS

CODE	BRAND NAME	MANUFACTURER
A	Deelastic	Dee Division of Handy & Harmon, Chicago, Ill.
C_1	Plasticoll	Lee S. Smith & Son, Pittsburgh, Pa.

In Part I, impression material A was selected. The manufacturer states: "Immersion in a dilute solution of potassium sulphate (about 2%) until pouring the model will improve the results."

Previously prepared hydrocolloid was stored in a water bath at 140° F. for at least 20 minutes. The trays were loaded from material in the storage bath and placed in a tempering bath for 3 minutes at 102° Fahrenheit. (A thermometer imbedded in the center of the mass of material in the tray showed the inter-

nal temperature to be 108° F. following this 3-minute tempering.) Small injection syringes were used direct from the 140° F. storage bath for injecting the material into the depth of the preparation and completely surrounding the porcelain tooth (Fig. 2). The tray was then carried to place and the entire assembly of block, preparation, and tray immersed for 5 minutes in 4 quarts of tap water which averaged 70° Fahrenheit (Fig. 3). This procedure was carried out at room temperature which averaged 75° Fahrenheit. From each impression, which was placed in 2 per cent potassium sulfate for 15 to 20 minutes, a model was poured in artificial stone* (Figs. 4, 5).

Fig. 2.—Material from small syringe injected into cavity preparation and surrounding tooth ready for placing of loaded tray.

Fig. 3.—Assembly to be chilled in 4 quarts of water 70° F. for 5 minutes.

A wax pattern, made on the stone model and not referred to the original preparation, was reproduced in hard dental gold, and an accurately fitting casting for each of the three preparations was obtained. No adjustment of the casting was considered allowable (Figs. 6, 7 and 8). The fit of these castings to subsequently made models was the criterion for accuracy of the models.

*S.S.W. Albastone.

Part 2

In testing, the above impression procedure was followed in all cases. Where water only was recommended by the manufacturer, the impression was not placed in potassium sulfate.

Fig. 4.—Separated impression.

Fig. 5.—Poured die.

MATERIAL A TESTS

Two accurate impressions of each preparation were made; one was sealed in a jar of tap water, one in a similar jar of freshly prepared 2 per cent potassium sulfate solution as follows: 1. Nov. 23, 1947, 2. Dec. 6, 1947, 3. Dec. 20, 1947, 4. Jan. 3, 1948, 5. Jan. 17, 1948, and 6. Jan. 24, 1948.

The 36 dies were all poured Jan. 31, 1948, in the following manner: Small amounts of artificial stone* from the same supply were mixed to puttylike consistency and vibrated into the depths of the impression from the point of a small instrument. A small ball of stone was added to aid in removal of the die

*S.S.W. Albastone.

from the impression. Each poured impression was then immediately replaced in the jar of water or potassium sulfate solution, in which it had previously been stored, for the period of the stone set. After one hour, allowed for setting of the diet, the dies were removed and mounted in display rings. The mounted dies

Fig. 6.—M-O inlay in porcelain tooth. (This and other illustrations of the castings are made with 13.5 diopters magnification added to a 3.5 lens, reducing a 14-inch focal length to 2 inches. This method of magnification reduces depth of focus but is necessary for the desired marginal detail. Defects not visible to operating loupes become evident in this manner.)

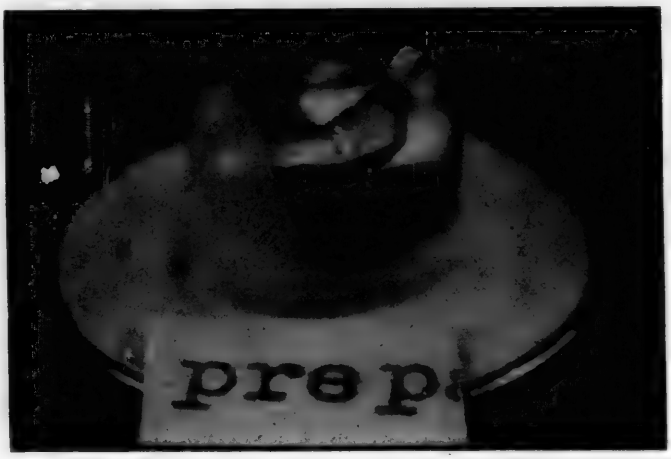


Fig. 7.—Three-quarter crown on porcelain tooth.

were now allowed 24 hours for complete drying out before any testing of the castings to the dies was made. These dies of each porcelain-tooth preparation gave examples of results from impressions stored in water, and 2 per cent potassium sulfate solution, respectively, for periods of 1, 2, 4, 6, 8, and 10 weeks.

To check upon the accuracy of the method, impressions of each preparation were made and run immediately, the stone then being placed in water and 2 per cent potassium sulfate solution, respectively, for the period of set.

Another series of impressions was made and placed in water and 2 per cent potassium sulfate solution for 15 minutes before pouring the dies and then replaced in their respective solutions for the period of stone set. These two series covered the usual time intervals occurring in the author's office, and the dies then acted as controls, since all castings fitted their respective dies accurately in these two series.

Fig. 8.—Mesio-occluso-distal with disto-lingual veneer on porcelain tooth.

Observations

It is important to understand that with the straight-sided perforated trays used any oversize in a die means the impression material has contracted. All the dies were oversize, so that it was impossible to seat any casting. The material between the lumen of the impression and the straight sides of the tray, into which it was locked by the perforations, had contracted in the interval between making the impression and pouring the model. Those impressions which had been in 2 per cent potassium sulfate solution appeared to have contracted slightly more than those stored in water, making greater oversized dies. The greatest discrepancy was observed in the dies of the complex M-O-D-L preparation.

The same impression procedure was again followed, storing the impressions in their respective solutions for periods of (1) 40 hours, (2) 24 hours, (3) 18 hours, and (4) 12 hours.

In this series the dies again were too greatly oversize to permit the fit of any of the castings, with the greatest discrepancy in the most complex preparation. There appeared to be a slightly decreasing degree of enlargement of the die as the time between making the impression and pouring the die was shortened.

Impressions were then made of each tooth and stored similarly for four hours before pouring the dies. In the four-hour series too great contraction had occurred to allow the castings to go to place upon the oversize dies, with the greatest discrepancy again in the most complex preparation (Figs. 9 and 10).

The procedure was repeated for a two-hour period and the dies found to be accurate, allowing the castings to seat perfectly (Figs. 11 and 12).

It therefore was determined that the distortion occurred at a point somewhere between the two- and four-hour storage intervals.

The procedure was repeated with a three-hour storage of the impressions in their respective solutions before running the dies. Just perceptible discrepancies were observed in this series. The M-O cavity preparation showed a slight opening at the gingival margin; the three-quarter crown casting and the M-O-D-L castings would go to place upon the dies but distinct frictional resistance was detectable.

Fig. 9.—Material A. Die poured after 4 hours' storage in water.

It was noted that distortion of dies of series stored less than 12 hours was less in K_2SO_4 (2 per cent) than in water. At 12 hours the distortion was approximately the same. Beyond 12-hour storage the distortion was greater in 2 per cent K_2SO_4 than in H_2O and the difference was progressively greater with increased storage time.

MATERIAL C_1 TESTS

Methods: Since the manufacturer recommends only water or wrapping in a wet cloth for storage of impressions of this material between making the impression and pouring the model, the potassium sulfate storage was omitted. The same impression procedure was followed.

Observations

Impressions with this material gave poor results. The conditions appear to conform to the manufacturer's temperature requirements but the impressions made and poured immediately, then placed in water for the period of stone set, appeared to have some lateral expansion, the proximal buccal and lingual margins between casting and constricted or undersized die being slightly open on successive tests. Impressions stored in water for 15 minutes before pouring gave a distinct expansion laterally of the impression with a constricted or undersize die which allowed definite rocking of the three-quarter crown and the M-O-D-L casting.

Fig. 10.—Material A. Die poured after storage 4 hours in 2 per cent potassium sulfate solution.

Impressions of this material, made and stored in water for 30 minutes, 1 hour, 90 minutes, and 2 hours, produced markedly undersized dies in each case. In Table II are shown the time limits accuracy was maintained.

TABLE II

TIME LIMIT ACCURACY IS MAINTAINED BETWEEN MAKING IMPRESSION AND POURING DIE

MATERIAL	MATERIAL STORED IN:	
	K_2SO_4(2%)	TAP WATER
A	2 to 3 hours	2 to 3 hours
C_1		0

It is evident that impressions of material C_1, the one which shows expansion, would of necessity have to be poured immediately if usable results could be attained.

Satisfactorily hard and smooth die surfaces were obtained with both materials, whether poured immediately or deferred over a period of weeks, and whether stored in 2 per cent K_2SO_4 or in tap water. This is contrary to the com-

monly reported experience. All impressions were partially dried with compressed air before the stone was introduced. Apparently this dispersed sufficiently the exudate from the material which is reported to have deleterious effects upon stone die surfaces.[2]

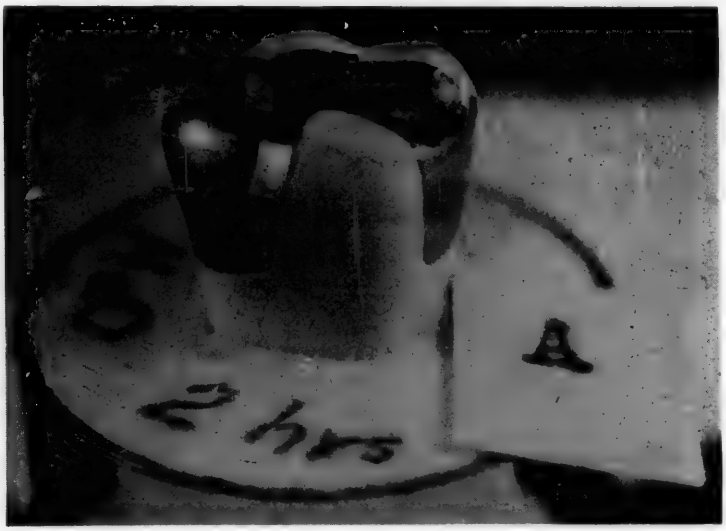


Fig. 11.—Material A. Die poured after 2 hours' storage in water.

DISCUSSION

Heretofore tests for the accuracy of a reversible hydrocolloid impression material have largely been based upon the reproduction of geometric models with pegs representing teeth. Accuracy is then tested either by micrometric measurements[2] or by the fit of a large casting made to fit the master model. The micrometric measurements, while accurate for linear change, are difficult to translate into terms of effects on inlay preparations. Micrometric measurements on stone dies were found impractical, as even with specially constructed gold castings for the spindle and anvil of the micrometer, each adjustment of the micrometer to the die resulted in surface abrasion of .002 to .003 inch or more. This precluded obtaining any accurate series of measurements from which to obtain an average. Slight give or spring in the castings over large areas is very slightly discernible and tensions in the castings almost undetectable.

The use of typical inlay or crown preparations with definite margins allows close and accurate examination of the fit of the casting. The relative rigidity of the casting precludes any distortion. Furthermore, slight frictional resistance may be detected through the fingers when the casting is put on and taken off the die.

While it has been suggested that slightly oversized dies might be used to compensate for the shrinkage of gold in casting, it appears inconceivable that

shrinkage or contraction of the impression material could occur without distortion, because of the varying thickness between lumen and tray wall.

It must be borne in mind that these tests have been made on materials available at the time of the tests and are only for those particular materials. Changes in formulas may be made or may have been made since these tests were conducted.

Fig. 12.—Die poured after 2 hours' storage in 2 per cent potassium sulfate solution.

Experience with full arch impressions of articulating models which had been stored in 2 per cent potassium sulfate solution for periods of 6 months to 9 months had indicated the probability of accuracy of the impression being maintained over long periods. Models made from these variously stored impressions appeared to articulate interchangeably with accuracy. The experience with the small, accurate castings with easily observable marginal contacts, however, shows the fallacy of relying upon gross observations. More adequate knowledge of the properties of reversible hydrocolloid impression materials is necessary. Certain reversible hydrocolloids have been found to be accurate in all operative procedures requiring precision when properly used. I believe, however, that the manufacturers of such impression materials should state the properties of their respective products and, even more important, the limitations existing. I also believe that dentists not employing technicians in their own offices will find far more satisfactory results if they will pour their own models shortly after making impressions. *Under no circumstances should the impressions be sent out of the office for pouring.*

SUMMARY AND CONCLUSIONS

1. A method of testing accuracy of impressions made with reversible hydrocolloid materials immersed in solutions for varying time intervals has been described.

2. Two stock materials were tested. One contracted at a critical end-point interval of time; one material expanded during the interval of stone setting. No change in accuracy of the controls was noted.

3. Dies poured from these contracted impressions are expanded and prevent test casting from fitting accurately. Dies poured from the expanded impressions are undersize and prevent accurate fitting of the test casting.

4. All hydrocolloid impressions should be poured immediately. Under no circumstances should they be immersed in solutions or wrapped in a wet towel and sent out to a commercial laboratory for pouring.

5. Immersion of impressions in potassium sulfate does not maintain them in equilibrium any more than immersion in water after the critical end point has been reached.

6. Accuracy is possible only if hydrocolloid impression material is handled properly.

REFERENCES

1. Skinner, E. W., and Kern, W. R.: Colloidal Impression Materials. V, *J. A. D. A.* **25**: 578, 1938.
2. Paffenbarger, G. C.: Hydrocolloidal Impression Materials: Physical Properties and a Specification, *J. A. D. A.* **27**: 386, 1940.

MAINTENANCE OF EQUILIBRIUM IN REVERSIBLE HYDROCOLLOID IMPRESSIONS

II. Made With Materials Supplied by the Manufacturers for Testing

ALLISON G. JAMES, D.D.S.

Beverly Hills, Calif.

INTRODUCTION

THE procedure used in testing stock materials has been described.* The fit of test castings to dies produced from impressions made with these materials was the criterion of accuracy. This paper describes the results obtained with materials supplied by the manufacturers for testing, using the same procedures and the same test castings.

MATERIALS AND METHODS

Table I gives the materials tested, identified by their code letters, together with the manufacturer's name.

TABLE I

MATERIALS

CODE	BRAND NAME	MANUFACTURER
A_1	Deelastic-special	Dee Division of Handy & Harman, Chicago, Ill.
A_2	Deelastic-gray	
B	D P Impression Colloid and Syringe Material	Dental Perfection Company, Glendale, Calif.
C	Plasticoll	Lee S. Smith & Son, Pittsburgh, Pa.

Material A_1 was prepared by the manufacturer to provide easier flow from the small injection syringe with no reduction in strength. Material A_2 was supplied in a gray color for possible greater visibility in the depth of impressions, but was of the same formula as material A, reported previously.*

Material B, supplied by the manufacturer, consisted of two materials: stock "Impression Colloid" for use in the tray, together with a special material, prepared for the small syringe, used to inject the deep portions of the cavity. The tray material had insufficient flow to be usable in a small syringe.

Material C was stated to be sample of the manufacturer's regularly marketed product. All materials were tested in the same way, except that only the A materials were immersed in potassium sulfate, as well as water. The B and C materials were tested only in water.

Impressions of each of the three porcelain tooth preparations were made, as described previously,* with each material and poured immediately. The dies produced from these impressions then acted as controls.

Impressions of each porcelain tooth preparation were made again with each of the materials and immersed in water for 15 minutes prior to pouring the models. Additional impressions with materials A_1 and A_2 were made and immersed in 2 per cent potassium sulfate solution for 15 minutes. The 15-minute

Received for publication June 14, 1948.

*James, A. G.: *J. D. Res.* 28: 108, 1949 (this issue).

storage of impressions (between making and pouring the models) was included as part of the establishing of controls, because this range of time covers the usual time intervals occurring in the author's office. Since the complex M-O-D-L preparation gave the best test for accuracy, all testing of materials beyond the 15-minute storage time was carried forward with that preparation alone.

MATERIALS A_1, A_2 TESTS

The manufacturer stated: "Immersion in a dilute solution of potassium sulphate (about 2 per cent) until pouring the model will improve the results."

The test castings fitted accurately the dies produced with both these materials from impressions poured immediately and from those stored for 15 minutes, one series in tap water and one series in 2 per cent potassium sulfate. The two materials reacted identically and appeared to have similar flow. Impressions of the M-O-D-L preparation were made and stored, one in 2 per cent potassium sulfate and one in tap water for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hours.

Those impressions stored in 2 per cent potassium sulfate solution gave accurate dies only up through two hours, marked oversize dies resulting from all potassium sulfate storage beyond that point.

Impressions with these materials stored in water gave accurate dies up through seven hours; the dies from impressions stored beyond this time were oversize. This lessened stability of the material in 2 per cent potassium sulfate solution, and increased stability in water may be attributable to a change in formula, but also the question of age of the material may be important.

MATERIAL B TESTS

The manufacturer of this material requests that only water be used for storage of the impression between its making and pouring the model.

Impressions of each porcelain tooth preparation made and poured immediately, and those made and stored in water 15 minutes, produced dies of complete accuracy for all testings.

Impressions of the complex M-O-D-L preparation were now made and stored in water for periods of 1, 3, 4, 15, 16, 17, and 18 hrs.

Impressions immersed in water up through fifteen hours gave accurate dies. There was slight frictional resistance observable in the fit of the casting to the 16-hour die, an enlargement observable in the 17-hour die (Fig. 1) and a marked enlargement in the 18-hour die (Fig. 2).

An interesting observation is the sudden change seen in the fit of the casting to the dies, a difference of immersion time of one hour producing a totally different die.

MATERIAL C TESTS

The manufacturer of this material recommends only water or wrapping in a wet cloth for preserving the impression between making it and pouring the model. The dies from the impressions poured immediately were slightly undersize, similar in every respect to those produced from impressions made with the

open market material described previously.* The impressions stored in water for 15 minutes, prior to pouring the models, had definite expansion with a resulting undersized die which allowed rocking of the three-quarter crown and M-O-D-L test castings (Fig. 3). Since this material appeared identical in every way with that purchased on the open market, it seemed needless to carry the investigation further.

Fig. 1.—Material B. Die poured after 17 hours' storage in water.

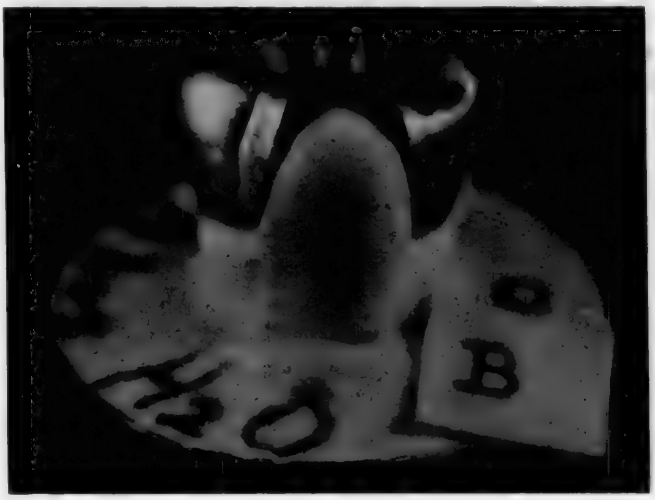


Fig. 2.—Material B. Die poured after 18 hours' storage in water.

In Table II are shown the time limits accuracy was maintained between making the impression and pouring the dies.

*James, A. G.: *J. D. Res.* 28: 108, 1949 (this issue).

TABLE II

TIME LIMIT FOR WHICH ACCURACY MAINTAINED BETWEEN MAKING IMPRESSION AND POURING DIE

MATERIAL	MATERIAL STORED IN:	
	K_2SO_4 (2%)	TAP WATER
A_1	2 hours	7 hours
A_2	2 hours	7 hours
B		16 hours
C		0 hours

DISCUSSION

The wide variation of time in which stability may be maintained between the impression making and the pouring of the model, especially as this relates to manufacturers' samples and open market samples of the same material, indicates variables which may be due to age of the material. This should be further investigated.

Tap water, rather than distilled water, was used as more representative of the actual conditions under which hydrocolloid impression materials are handled in dental offices throughout the country.

Fig. 3.—Material C. Die poured after impression stored in water 15 minutes. This is only material which expanded, giving an undersize die.

The author believes that the use of such test castings as described produces a more practical method for testing the accuracy of impression materials than has heretofore been used, and may easily be applied by any dentist interested in determining the actual properties of the impression material he uses in his practice. The experiences with impression materials further point out the value to the manufacturer as well as to the dentist which would ensue were the manufacturer to state positively the limits of accuracy for his impression material.

This would materially assist in reducing the criticism and disappointments which have occurred in the use of reversible hydrocolloid impression materials. While the manufacturers all recommend that the impression be poured immediately, they nevertheless imply that no damage to the impressions may occur if the pouring of the model is postponed, and the impression "preserved" according to their directions.

These tests are only for the particular material tested and not necessarily for similar materials with different formulas.

SUMMARY AND CONCLUSIONS

1. Four materials supplied by manufacturers for testing were tested for accuracy of impressions made according to methods previously described.

2. All materials, except one material which expanded, contracted at a critical end-point interval of time. This end point varies with the material tested and possibly with the shelf life. No change of accuracy was noted in the controls.

3. Dies poured from those contracted impressions were expanded and prevented test castings from fitting accurately. Dies poured from the expanded impressions were undersize and likewise prevented accurate fit of the test castings.

4. Immersion in 2 per cent potassium sulfate solution of impressions made with materials for which potassium sulfate is recommended did not maintain them in equilibrium as long as did immersion in water.

5. All reversible hydrocolloid impressions should be poured immediately if possible, but never after the critical point established for the material used.

6. Complete accuracy is possible, but only if hydrocolloid impression material is properly handled.

REFERENCES

1. Skinner, E. W., and Kern, W. R.: Colloidal Impression Materials. V, *J. A. D. A.* 25: 578, 1938.
2. Paffenbarger, G. C.: Hydrocolloidal Impression Materials: Physical Properties and a Specification, *J. A. D. A.* 27: 386, 1940.

THE EFFECT OF WETTING AGENTS ON ORAL LACTOBACILLI

II. Local and Systemic Toxicity in Mice. Toxicity vs. Antibacterial Activity

FRANK E. WALTER, D.D.S.,* AND HARVE J. CARLSON, Dr.P.H.†

Department of Microbiology and Pediatrics, School of Medicine, Western Reserve University, Cleveland, Ohio.

SYNTHETIC surface-active agents[1] were introduced into industry at the time of World War I because of a unique combination of physical assets; namely, wetting, penetrating, emulsifying, dispersing, and foaming properties. They have recently been introduced to the general public as the active ingredient in the new detergents, hair washing preparations and dentifrices. However, their most remarkable properties are not physical but chemical and electrolytic. The surface-active agents are anionic, cationic, or nonionic when in solution. This activity depends upon the relative balance of the two atomic groups within a molecule. The anionic agents ionize so the major part of the molecule is the anion. These substances are chiefly sulfonates or sulfates. The cationic agents ionize so the major part of the molecule is the cation and they are substituted ammonium or cyclic quaternary ammonium compounds. The nonionic agents do not ionize when dissolved in water. These are polyethylene glycol fatty acid esters or ethers of alkyl aryl alcohols and polyoxalkaline derivatives of hexitol anhydride fatty acid esters. It is to be noted that a more definite chemical classification of these agents is impossible since the majority of commercially available substances are impure or of secret or patented formulae. There is apparently no limit to the possibility of variation within the molecules, but the anionic and cationic types are similar in that each is a large polar molecule composed of two distinct atomic groupings, one of which is lipophilic and hydrophobic and the other is lipophobic and hydrophilic.

It is because of the latter properties that these substances have the ability to influence profoundly the permeability of cell membranes. However, this ability to influence the cell membrane was unrecognized for a number of years and therapeutic application is in an embryonic state of development at the present time.

The term "wetting" is commonly used to describe these substances, but in a strict sense this is incorrect since it refers to only one of the five physical properties which Fischner[2] describes in more detail as follows: (1.) wetting—producing greater spread of a liquid over a repellent surface; (2.) penetrating—speeding passage of a liquid into a porous solid; (3.) emulsifying—stabilizing emulsions; (4.) dispersing—stabilizing suspensions of solids in liquids; (5.) foaming—stabilizing gas-liquid relationships.

It is obvious that a combination of these properties would be very advantageous in any substance under consideration for possible therapeutic application in the control of caries. On the basis of these potentialities a large

This work was done at the Babies and Children's Hospital, Cleveland, Ohio, and was aided in part by a grant from the Procter and Gamble Company.

Received for publication, July 26, 1948.

*Teaching Fellow in Bacteriology, School of Dentistry, Western Reserve University.

†Assistant Professor of Bacteriology, School of Medicine, Western Reserve University, Director of Laboratories, Babies and Children's Hospital, Cleveland, Ohio.

group of surface-active agents was screened and a preliminary report published (*J. D. Res.* **26**: 353, 1947).

In the consideration of the surface-active agents as possible therapeutic substances in the control of dental caries it is essential that the toxicological properties of the substances be known. Woodard and Calvery[3] have evaluated the factors to be considered in a study of the toxicity of surface-active agents. A review of the literature[4] has indicated that sensitization phenomena are rarely encountered in the use of these agents. For this reason, and because of the difficulty of accurately ascertaining the sensitizing ingredient in many of the commercial products, a study of the possible allergic phenomena has not been included. In this paper, an attempt was made to correlate the antibacterial effect of various classes of surface-active agents with the local and systemic toxicity as indicated by mouse inoculation.

METHODS

Anionic, cationic, and nonionic surface-active substances were used in the study. The substances, regardless of the percentage of active ingredient in the commercially available product, were tested in 1:100 and 1:1000 dilutions in distilled water. The dilution was made by weight/volume with solids and by volume/volume with liquids. After dilution the solutions were sterilized by autoclaving at 121° C. for 15 minutes. The pH of each solution was determined* before and after sterilization and at the end of the test series. Throughout the study the solutions were handled aseptically to avoid possible changes as the result of resterilization. One-half milliliter of each solution was injected intraperitoneally into young adult white Swiss mice varying in weight from 20 to 40 grams. Injections were made daily, alternately in right and left lower abdominal quadrants, for 10 successive days.

During the test period, daily observations were made just prior to inoculation. An evaluation of the general appearance was made, including (a) condition of the fur, eyes, muscle tonus, and irritability, (b) gastrointestinal symptoms, including anorexia and diarrhea, and (c) local reaction at the site of inoculation. Autopsies were conducted upon animals which died during the test period when examination could be carried out within approximately two hours of death. No attempt was made to evaluate conditions present in animals dead longer than this period of time. The autopsy included (a) examination of the site of injection, especially for the presence of inflammation, necrosis, cicatrization, and adhesions, (b) gross examination of all viscera, especially the liver, kidneys, and spleen, and (c) microscopic examination of tissues from organs showing gross changes, especially inflammation or hemorrhage. At the end of the test period, half or more of the animals surviving inoculation of an agent were sacrificed. Autopsies were conducted upon this group in the manner previously described.

At the conclusion of the tests the toxicities were correlated with the previously determined activity of the agents against *Lactobacillus acidophilus, Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*.[5, 6] For purposes of clarity the correlations are tabulated (see Tables I-VII) according to the classification of the surface-active substances.

*Beckman pH meter.

TABLE I. TOXICITY OF SURFACE-ACTIVE AGENTS

NAME	FORMULA	CONC.	pH	A	B	C	L	K	I	GI	RF	r
ANIONIC AGENTS												
A. *Alkyl sulfates*												
Arctic Syntex M	mixed fatty acid monoglyceride sulfate		6.5	10	0		-	-	-	-	-	
Duponol PC	lauryl alcohol sulfate		7.7	3	3	7	-	+	-	-	-	
B. *Alkyl sulfate, sodium salt*												
Orvus WA	hymolal with sodium lauryl sulfate		5.4	4	0		-	-	-	-	-	
Tergitol Penetrant 4	sodium tetradecyl sulfate	25	4.5	10	10	10	+	+	-	-	.	
Tergitol Penetrant 7	sodium heptadecyl sulfate	25	9.4	4	2	7	-	+	-	-	-	
C. *Alkyl sulfate, ammonium salt*												
Tergavon C	fatty acid sulfate-ammonium salt		5.75	4	1	7	-	-	-	-	-	
D. *Alkyl sulfate substituted amide*												
Michelene DCA	sulfated glyceryl amide		9.6	10	0		-	-	.	-	-	
E. *Alkyl ester sodium sulfate*												
Aerosol 18	n-octadecyl disodium sulfosuccinamate	35	2.75	6	6	6	-	±	-	+	-	
Aerosol MA	di-hexyl sodium sulfosuccinate	100	4.2	6	6	4	+	-	-	+	-	
Aerosol AY	di-amyl sodium sulfosuccinate	100	3.1	10	0		-	-	-	-	-	3
Aerosol OT	di-octyl sodium sulfosuccinate	100	4.0	10	1	10	-	-	.	-	-	3 3
Aerosol 1 B	di-isobutyl sodium sulfosuccinate	100	3.2	10	0		-	-	-	-	.	3
Aerosol 22	n-octadecyl tetrasodium n (1, 2 dicarboxyethyl sulfosuccinamate)	95	7.0	10	10	1	+	+	.	.	.	
F. *Alkyl sulfonate*												
Sulfonated castor oil	sulfonated castor oil		8.0	10	6	5	-	-	10	2	-	2

Legend:

pH = pH of 1 per cent solution.
A = number of animals tested.
B = number of animals died.
C = number of test days in which deaths occurred.
L = liver damage.
K = kidney damage.
I = local toxicity.
GI = gastrointestinal toxicity: diarrhea.
RF = rough fur.
+ = positive pathological finding. May be replaced by 1-10 to indicate number of affected animals.
- = negative pathological finding.
r = note.

Notes:

1 = CNS paralysis in 10 minutes.
2 = lethargic, abdominal distention.
3 = alert, sleek.
4 = highly toxic.
5 = survivors appeared fully recovered in 2 days.

G. *Alkyl ester sulfonate*												
Arctic Syntex A	sulfonated oleic acid ester	85	6.5	10	0		-	-		-	-	
Nacconol LA	lauryl sulfoacetate		5.0	10	0		-	+	5	5	+	
Sandozol N	sulfonated vegetable ester		2.75	5	0		-	-	-	-	+	
Surfax W. O.	sulfonated fatty ester		5.8	10	0		-	-	-	7	+	
H. *Alkyl sulfonate substituted amide*												
Arctic Syntex T	substituted amide		7.25	4	0		-	-	-	-	-	
I. *Aryl sodium sulfate*												
Stablex A	sodium naphthalene sulfate		6.8	10	3	1 hour						
Stablex B	naphthalene		6.8	10	10	8	+	+	-	-	-	
J. *Alkyl aryl sodium sulfate*												
Penetrant 1000	sodium alkyl aryl sulfate		6.9	6	4	6	-	-	-	-	-	1
K. *Alkyl aryl sulfonate*												
Aheowet ANS	alkyl aryl sulfonate		5.75	10	10	3	+	+	-	-	-	2
Cerfak NS	alkyl aryl sulfonate		6.0	9	0		-	-		-	-	
Quaker Dianol 11	alkyl aryl sulfonate		6.0	10	0		-	-				
Solvadine NC Conc.	alkyl aryl sulfonate		6.9	10	4	9	1	1	-	1	-	
Surfax TR	alkyl aryl sulfonate		8.2	4	1	4	-	-	-	-	-	
L. *Alkyl aryl sodium sulfonate*												
Aerosol OS	isopropyl naphthalene sodium sulfonate		5.1	4	4	3	±	±	-	-	-	
Nacconol NR	sodium alkyl aryl sulfonate	40	3.6	4	0		-	-	-	-	-	
Supersulfate F. S. Powder	sodium alkyl aryl sulfonate		7.0	4	0		-	-	-	-	-	
Triton X-200	sodium alkyl aryl polyether sulfonate (mixture)	28	4.3	10	0		-	-		-	-	3
Ultrawet E	85% alkyl aryl sodium sulfonate 15% sodium sulfate		7.1	10	8	4	+	-	-		-	5
Ultrawet 30 DS	same as above		4.2	10	4	6	-	+	-		-	
M. *Petroleum sulfonate*												
Stablex G	petroleum sulfonate		6.3	4	2	6	-	-	-		-	
N. *Petroleum sulfonate, sodium salt*												
Acto 500	sodium petroleum sulfonate	50	6.0	10	5	6	-		-		-	
Acto 550 W	sodium petroleum sulfonate	57	6.5	10	5	6	±	±			-	
Acto 600	sodium petroleum sulfonate	60	6.6	4	4	10	-	-		-	-	
O. *Unclassified*												
Aheowet RS	alkyl naphthalene sulfate deriv.		6.5	10	10	4	+	+	-	-	-	
Cerfak F	sulfonated amide		4.2	5	0		-	-	-	±	-	
Detanol P	higher sulfonated fatty acid deriv.		6.0	9	0		-	-	-	-	-	
Detanol P 26	same as above		5.5	10	0		-	-		-	+	

TABLE I. TOXICITY OF SURFACE-ACTIVE AGENTS—(CONT'D)

NAME	FORMULA	CONC.	pH	A	B	C	I	K	l	GI	RF	r
Duofol L	sulfated condensation product		2.6	5	0		-		-	-	+	
Glyco DS*	diglycol stearates	100	6.0	10	0		-	+	-	+	+	
Glyco TES†	trihydroxyethylamine stearates, special		7.5	10	0		-	-	l	5	5	
Igepon T	substituted amide		5.0	10	0		-	-	-	-	-	
Ninol 737	fatty acid alkanolamine	99	8.8	6	2	8	-	-	-	-	-	
Petronate	$C_{26}H_{25}SO_3Na$		6.4	4	4	4	-	+	-	-	-	
Quaker Dianol 20	ester sulfate		5.5	8	2	3	-	+	-	-	-	
Resolin B	substituted hydrocarbon		6.9	4	4	2	+	+	-	-	-	
Soapotol	higher fatty acid with sodium sulfate, triethanolamine and oleic acid		6.5	10	1	6	-	2	-	-	+	
Victawet 35 B	$Na_5R_5(P_3O_{10})_2$		6.5	4	4	2	-	+	-	-	-	
Victawet 54 B	same as above		6.1	4	2	5	-	-	-	-	-	
PREIONIC AGENTS												
Alox 325	complex methyl esters		8.6	3	2	10	-	-	-	-	-	
CATIONIC AGENTS												
Alkaterge O	substituted oxazoline	100	7.5	10	5	9	-	-	-	-	-	
Aerosol C 61	ethanolated alkyl quanidine amine		8.0	10	9	4						
					10	7	+	+	-	-	-	
Onyx	alkyl dimethyl benzyl ammonium chloride, batch 73		6.7	4	4	1	±	±	-	-	-	
Aerosol 1861	Aerosol 18 and C 61			10	2	10	-	-	+	+	+	
Sapamine KWC	diethyl aminoethyl oleyl amide acetate	99	2.5	10	10	2	+	+	-	-	-	
Softex AP			3.25	10	0		-	-	-	1	-	
Triton X-400	cetyl dimethyl benzyl ammonium chloride	25	3.9	10	10	1	+	+	-	-	-	4
	OR											
Victamine D	RNH—R=O ONH₂R		6.8	10	10	3	+	-	-	-	-	
NONIONIC AGENTS												
Cerfak N-100	fatty condensate	100	8.0	10	0		-	-	-	-	-	
Cerfak P	polyethylene glycol ester		6.1	10	1	10	-	-	-	+	-	
Cerfak 218	polyethylene glycol ether		5.5	10	10	5	-	-	-	-	-	1
Sonoquol	water-miscible mineral oil		7.4	10	1	10	1	1	-	-	-	
Triton X-30	alkyl aryl polyether alcohol	33	5.0	6	4	6	-	+	-	+	-	
Tween 60	polyoxalkalene ether of partial stearic acid ester	97+	3.5	10	0		-	-	-	-	-	3
	OR											
Victawet 12	R—O—R=O			10	10	10	4	4	-	+	-	
	OR											

*Glyco diglycol stearates.
†Glyco trihydroxyethylamine stearates, special.

RESULTS

Sixty-nine agents were tested. Of this number 53 were anionic, 8 were cationic, 7 were nonionic, and 1 was preionic.

Cationic agents.—The cationic agents were almost uniformly toxic when administered by injection as described. Only one (see Table I) proved to be nontoxic within the test period. Onyx, Sapamine KWC, and Triton X-400 were sufficiently toxic to cause death in all test animals in a maximum of 2 days or after 2 intraperitoneal injections of 0.5 ml. of 1 per cent concentration. Autopsies revealed kidney and liver damage in all animals. Onyx was retested in 0.1 ml. concentration by the same procedure and produced death in 3 of 10 animals within 6 days. All surviving mice showed gross kidney damage at autopsy. The pH of the 0.1 per cent solution was 6.5. Administration of Victamine D and Aerosol C 61 resulted in the death of all test animals within the 10-day period, and autopsy revealed that the former agent produced liver damage and that the latter produced liver and kidney lesions. The other cationic agents, with the exception of Softex AP, were toxic to some degree. Softex AP was not lethal within the limits of the test nor did it produce local or general toxic signs. The animals were sleek and healthy-appearing at the end of the test period. The composition and per cent active ingredient of this agent are not known at this writing. The activity of these cationic agents against *L. acidophilus, Staph. aureus, Esch. coli, P. vulgaris* and *Ps. aeruginosa* is summarized in Table II. With the exception of Onyx no activity was displayed against *Esch. coli* and *P. vulgaris* in the technic used. Onyx was the most active inhibiting agent against *L. acidophilus* but it was also the most toxic. Triton X-400 and Onyx exhibited bactericidal activity against *L. acidophilus* when used in dextrose broth cultures, at slightly acid pH. One-tenth milliliter aliquots were subcultured onto tomato juice agar at intervals up to 48 hours with negative results.

TABLE II

ACTIVITY OF CATIONIC AGENTS AGAINST BACTERIA (OXFORD CUP METHOD)

NAME	L. ACIDOPHILUS				STAPH. AUREUS	ESCH. COLI	P. VULGARIS	PS. AERUGINOSA			
Dilution	10^{-1}	10^{-3}	10^{-5}	10^{-6}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-5}	10^{-6}
Alkaterge O	p20	sa	s15	x	x	x					
Aerosol C 61	x	x	x	x	c14	x					
Onyx	c35-p40	c15	ca	s	c30*	c16*	p	s44	x	x	x
Sapamine KWC	pa	pas	s+-	s-	x	x					
Softex AP	ca	pa	pas	pas	x	x					
Triton X-400	c12	ca	x	x	x	x					
Victamine D	pa	pa	-	-	-	-					
Aerosol 1861	c14s	x	s	s	c18	x	-	x	x	x	x

Legend:

$10^{-1\ -3\ -5\ -6}$ = dilution of test substance in sterile distilled water.
p = partial inhibition.
c = complete inhibition in area denoted in millimeters.
a = cup area.
s = stimulation.
x = indefinite result or no activity.
- = data incomplete.
* = media change in cup area and adjacent to it.

To summarize, the cationic agents, with the exception of Softex AP, were highly toxic by intraperitoneal injection in the technic used. As may be noted in Table I, there is no apparent correlation between toxicity and pH of the cationic agents tested. In unpublished studies, it was found that Onyx produced a partial inhibition of *P. vulgaris* and stimulated the growth of *Ps. aeruginosa* when used in 1:10 concentration by the Oxford agar test cup method.

Nonionic agents.—(See Tables I and III.) There was insufficient information available to permit correlation of toxicity with chemical structure. Victawet 12, Triton X-30, Sonoquol, and Cerfak 218 proved significantly toxic. Cerfak P resulted in the death of one animal, and the survivors had some diarrhea without other toxic signs. Cerfak N-100 showed significant activity against *L. acidophilus, Staph. aureus, P. vulgaris,* and *Ps. aeruginosa,* while it was not toxic to animals in the technic used. By the agar cup procedure, Tween 60 in 10 per cent solution produced a stimulation of *L. acidophilus* immediately adjacent to the cup area with an area of inhibition peripheral to the zone of stimulation, and in 1/10 per cent concentration it inhibited from the cup area peripherally with no stimulation. It was apparently nontoxic to animals within the limits of the test procedure. A third phenomenon observed in the work with the nonionic agents was the effect of Cerfak 218, a polyethylene glycol ether. Within 10 minutes after inoculation with ½ ml. of a one per cent solution of this agent, the animals exhibited motor paralysis, preceded by a period of hyperirritability. At the onset of paralysis the animals were mentally alert. They responded to light and sound stimuli but not to pain (pin stimulation). Breathing became accelerated and more shallow than normal. The animals then became stuporous, but could be partially aroused when picked up. The position reflex remained present. The animals remained in the comatose state until death. The experiment was repeated, using younger mice (3 to 4 weeks old), and the same effect was produced but death occurred within a maximum of 24 hours.

TABLE III
ACTIVITY OF NONIONIC AGENTS AGAINST BACTERIA (OXFORD CUP METHOD)

NAME	L. ACIDOPHILUS				STAPH. AUREUS	ESCH. COLI	P. VULGARIS	PS. AERUGINOSA			
Dilution	10^{-1}	10^{-3}	10^{-5}	10^{-6}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-5}	10^{-6}
Cerfak N-100	p25	x	s	s	c30	x	c	ca	ca	x	x
Cerfak 218	s	s	s	s	p12	x	-	x	x	x	x
Cerfak P	p46	p30	p26	p20	p17	x	-	x	x	x	x
Sonoquol	x	x	x	x	x	x	-	-	-	-	-
Triton X-30	c30	cap	p	s	x	x	-	-	-	-	-
Tween 60	s18-c25	c12	ca	s	p12	x	-	c17	s++	p30	p+
Victawet 12	c23	ca	x	s	c20	x	ca	c19	ca	ca	ca

Legend:
$10^{-1\text{-}3\text{-}5\text{-}6}$ = dilution of test substance in sterile distilled water.
p = partial inhibition (followed by + to indicate degree).
c = complete inhibition in area denoted in millimeters.
a = cup area.
s = stimulation (followed by + to indicate degree).
x = indefinite result or no activity.
- = data incomplete.

TABLE IV

ACTIVITY OF ANIONIC AGENTS AGAINST BACTERIA (OXFORD CUP METHOD)

NAME	L. ACIDOPHILUS				STAPH. AUREUS	ESCH. COLI	P. VULGARIS	PS. AERUGINOSA			
Dilution	10^{-1}	10^{-3}	10^{-5}	10^{-6}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-5}	10^{-6}
Acto 500	cp22	cp20	p30	cps	c17	x	-	p11	x	x	x
Acto 550 W	c40	c30	c15	s+	x	x	-	-	-	-	-
Ahcowet ANS	c12	c28	ca	cas	p11	x	-	-	-	-	-
Ahcowet RS	c14	s	x	s+	c13	x	p	x	x	x	x
Aerosol 18	c18	c10	s	x	cp15	x	p	p	x	x	x
Aerosol MA	cap13	x	x	x	c12	x	x	p12	x	x	x
Aerosol AY	c30	ca	ca	x	x	x	c	x	x	x	x
Aerosol OT	p20	x	s	x	c14	x	-	x	x	x	x
Aerosol 1 B	c18	x	s	x	c13	x	p	x	x	x	x
Aerosol 22	p23	x	x	x	p20	x	p	x	x	x	x
Aerosol OS	c40	c20	x	x	x	x	c	-	-	-	-
Arctic Syntex A	c15	x	s25	s+	x	x	x	-	-	-	-
Arctic Syntex M	cap	cap	cap	x	c14	x	-	x	x	x	x
Arctic Syntex T	c25	c12	ca	c	x	x	p	-	-	-	-
Cerfak F	c40	c35	x	x	p12	x	-	x	x	x	x
Cerfak NS	c33	c11	s+	s	c14	x	-	x	x	x	x
Detanol P	x	x	x	x	-	-	-	x	x	x	x
Detanol P 26	p30	x	x	x	x	x	x	-	-	-	-
Duofol L	c40	c23	x	x	c14	x	p	x	x	x	x
Duponol PC	c25	c12	ca	ca	c20	x	p	x	x	x	x
Glyco DS*	c12	c15	x	x	x	x	-	-	-	-	-
Glyco TES†	x	x	x	x	pas	x	-	x	x	x	x
Igepon T	x	x	x	x	x	x	x	-	-	-	-
Laurel Supersulfate F.S.	c20	ca	ca	x	c14	x	-	x	x	x	x
Michelene DCA	c20	s	s	x	p10	x	p	c12	x	x	x
Nacconol NR	c13	c12	x	x	c17	x	-	x	x	x	x
Ninol 737	p25	p20	p20	p20	c13	x	-	p23	x	x	x
Orvus WA	c40	c30	p	x	x	x	-	-	-	-	-
Penetrant 1000	c40	c25	pa	s	c15	x	c	x	x	x	x
Petronate	c20	c28	ca	pa	x	x	-	-	-	-	-
Resolin B	c40	p30	p20	cp	c30	x	c	p12	x	x	x
Sandozol N	c30	c12	x	s	c11	c11	p	x	x	x	x
Soapotol	c30	ca	s	x	x	x	-	-	-	-	-
Solvadine NC Conc.	c23	ca	s	s	c18	x	c	ca	ca	ca	ca
Stablex A	c40	x	x	x	x	x	-	-	-	-	-
Stablex B	c25	ca	s++	s	x	x	x	-	-	-	-
Stablex G	c50	c25	pa	s	x	x	p	-	-	-	-
Sulf. castor oil	p35	ca	x	p28	c14	x	-	c18-s+++	x	x	x
Surfax W. O.	x	x	x	x	x	x	x	-	-	-	-
Surfax TR	c22	cap	ca	s+	x	x	x	x	x	x	x
Tergavon C	p33	p18	s++	s++	c25	s	p	x	x	x	x
Tergitol Penetrant 7	c15p	c12p	x	s	c13	p12	-	x	x	x	x
Tergitol Penetrant 4	x	x	x	x	c25	x	-	-	-	-	-
Ultrawet	c25	c10	s	x	c14	x	-	x	x	x	x
Ultrawet E	c40	c15	x	x	c17	x	-	-	-	-	-
Ultrawet 30 DS	c26	ca	s	x	c19	x	-	x	x	x	x
Victawet 35 B	c40	c18	p	s	c20	c12	-	x	x	x	x
Victawet 54 B	cap	cap	ca	ca	p20	x	-	x	x	x	x

*Glyco diglycol stearates.

†Glyco trihydroxyethylamine stearates.

Legend: $10^{-1\text{-}3\text{-}5\text{-}6}$ = dilution of test agent.
p = partial inhibition (+ indicating degree).
c = complete inhibition, measured in millimeters.
s = stimulation (+ indicating degree).
a = cup area.
x = indefinite result or no activity.
- = incomplete data.

To summarize, the nonionic agents tested were less toxic than the cationic agents and were more variable in the correlation of toxicity and antibacterial effect. Some agents of low toxicity proved to have good antibacterial activity.

Anionic agents.—(See Tables I, IV, V, VI, VII.) To aid in statistical correlation the agents were classified according to chemical types (see Table I). The alkyl sulfates Arctic Syntex M and Duponol PC varied in their toxic manifestations but corresponded closely in antibacterial effect, both being highly effective against *L. acidophilus*. As may be noted in Table VI, Duponol PC exerted a bactericidal effect. The sodium alkyl sulfates Tergitol Penetrant 4 and Tergitol Penetrant 7 produced liver and kidney lesions when administered in a final concentration of 0.25 per cent. Orvus WA was nontoxic; however, it is not a pure chemical entity. The ammonium alkyl sulfate, Tergavon C, stimulated the growth of *L. acidophilus* and two of three strains of Proteus. In 10 per cent concentration it was not bactericidal in effect upon *L. acidophilus*. The alkyl sulfate substituted amide Michelene DCA produced no signs of toxicity and was active against all test organisms except *Esch. coli* (see Table IV).

TABLE V

EFFECT OF ANIONIC AGENTS ON PSEUDOMONAS AND PROTEUS IN LIQUID MEDIA

AGENT	PS. AERUGINOSA	P. VULGARIS	AGENT	PS. AERUGINOSA	P. VULGARIS
Acto 500	-	+	Ninol 737		+
Aerowet RS	+	-	Orvus WA	+	+
Aerosol 18	-	-	Penetrant 1000	+	+
Aerosol MA		+	Petronate	+	+
Aerosol AY	-	-	Resolin B		+
Aerosol OT	+	-	Sandozol N		+
Aerosol 1 B	+	-	Solvadine NC Conc.		+
Aerosol 22		+	Stablex A		+
Aerosol OS		+	Stablex B	+	+
Arctic Syntex T		+	Stablex G		+
Cerfak F		+	Sulf. castor oil		+
Cerfak NS		+	Surfax W. O.		+
Detanol P		+	Tergavon C		+
Duofol L		+	Tergitol Penetrant 7		+
Duponol PC		+	Tergitol Penetrant 4		+
Glyco DS		+	Ultrawet E		+
Glyco TES		+	Ultrawet 30 DS		+
Laurel SS		+	Victawet 35 B	-	-
Michelene DCA		+	Victawet 54 B		+

Legend:
+ = growth present in subculture, verified by Gram stain.
- = no growth in subculture.
Agent dilution = 10^{-2}.
Incubation of broth + organisms + agent = 24 hours.
Incubation of streak plates = 18 hours.

Alkyl ester sodium sulfates. The effect on toxicity of increase in length of the carbon chain and chemical regrouping is illustrated in the Aerosols. Aerosol 1 B, di-isobutyl sodium sulfosuccinate, seemed nontoxic. Aerosol AY, di-amyl sodium sulfosuccinate, was also nontoxic. Aerosol OT, di-octyl sodium sulfosuccinate, resulted in one death, but the survivors appeared sleek and healthy and when sacrificed presented no abnormal autopsy findings. Aerosol

18, n-octadecyl disodium sulfosuccinamate, (35 per cent concentration) killed all animals in six days and lesions of the kidneys were demonstrable. Aerosol MA, di-hexyl sodium sulfosuccinate, resulted in the death of all test animals in 4 days. The animals were diarrheal and presented liver lesions on autopsy. Aerosol 22, n-octadecyl tetrasodium n(1,2 dicarboxyethyl sulfosuccinamate), killed all test animals with one injection, and autopsy revealed liver lesions in all cases. No positive correlation between toxicity and antibacterial activity was evident in this group of agents. Aerosol 22, which was very toxic, was only slightly effective against bacteria. Sulfonated castor oil, the alkyl sulfonate, produced local lesions at the site of injection in all animals.

Alkyl ester sulfonates. Seven animals inoculated with Surfax W. O. were diarrheal from the third day until the conclusion of the series, but all recovered in a 3-day period after conclusion of the test. The sulfonated esters caused no deaths and, with the exception of Nacconol LA, no gross lesions. One alkyl sulfonate substituted amide, Arctic Syntex T, produced no toxic signs and was highly effective against *L. acidophilus* and Proteus species. The aryl sodium sulfate, Stablex A, was highly toxic and of poor antibacterial activity. It caused death in all test animals within 3 days. In 3 of the animals, death occurred within one hour of inoculation. The effect was typical of central nervous system depression. The alkyl aryl sodium sulfate, Penetrant 1000, also produced a significant number of deaths. In the alkyl aryl sulfonates toxicity was minimal with the exception of Aheowet ANS and Solvadine NC Conc. The former produced kidney and liver damage and resulted in the death of all animals within 3 days. Solvadine NC Conc. was somewhat less toxic, killing 4 of 10 animals. It also caused kidney and liver lesions as demonstrated by autopsy. This agent was notably effective against *L. acidophilus*, *Staph. aureus*, Proteus species and *Ps. aeruginosa*. Aheowet ANS, though more toxic than Solvadine NC Conc., was not as effective against bacteria.

Alkyl aryl sodium sulfonates. Aerosol OS caused the death of all animals in 3 days. Autopsy revealed kidney and liver damage. The effect of this agent was similar to that produced by Aheowet RS. The other agents in this group were nontoxic with the exception of the Ultrawets which are mixtures containing 15 per cent sodium sulfate. The petroleum sulfonate, Stablex G, resulted in the death of half of the test animals in 6 days, but gross findings were obscure. The sodium petroleum sulfonates Acto 500, 550 W, and 600 were highly toxic but also effective against *L. acidophilus*. Acto 500 demonstrated bactericidal activity in 10 per cent concentration in broth against all test organisms except *Ps. aeruginosa* (Table VI).

To summarize, the toxicity of anionic surface-active agents has been found to become more pronounced with increase in length of the carbon chain within a homologous series. This supports previous investigations.[2, 7] In general, there seems to be no positive correlation between pH of the solutions and toxicity in animals.

TABLE VI

ACTIVITY OF 10% NUTRIENT BROTH DILUTIONS OF ANIONIC AGENTS BACTERICIDAL VS BACTERIOSTATIC ACTIVITY

NAME	PS. FL.		PS. AER.		P. VUL.		P. D.		L. 7	
	MINS.	HRS.	MINS.	HRS.	MINS.	HRS.	MINS.	HRS.	MINS.	HRS.
	3	24	3	24	3	24	3	24	3	24
Acto 500	+	-	+	+	-	-	+	-	+++	-
Aheowet RS	+	-	+	-	++	++	+	+	+++	+
Aerosol 18	+	-	+	+	-	-	-	-	++	+
Aerosol MA	-	-	-	-	-	-	-	-	+++	-
Aerosol AY	-	-	-	-	-	-	-	-	+	-
Aerosol OT	+	-	+	-	+	-	-	-	+++	-
Aerosol 1 B	-	-	-	-	+	+	-	-	+	-
Aerosol 22	+	-	+	-	+	-				
Aerosol OS	+	-	+	+	+	-	+		+++	-
Arctic Syntex T	+	+	+	++	-	-	+	+	-	+
Cerfak F	+	-	+	-	-	-	+	-	+	-
Cerfak NS	+	-	+	-	+	-	+	-	+++	-
Detanol P	+	-	+	-	-	-	+	+	-	-
Duofol L	+	-	+	+	+	-	+	-	++	-
Duponol PC	+	-	+	+	-	-	-		-	-
Glyco DS	+	-	+	-	+	+	+	+	++	+
Glyco TES	+	+	+	-	+	-	+	-	+	-
Laurel SS	+	-	+	+	+	-	+	-	++	-
Michelene DCA	-	-	+	+	+	-	+	-	+++	-
Ninol 737	+	-	+	+	+	-	+	-	-	-
Orvus WA	+	-	+	-	-	-	+	-	-	-
Penetrant 1000	-	-	-	+	+	-	+	-	+--	-
Petronate	+	-	+	+	+	-	+		++	-
Resolin B	+	-	+	+	+	-	+	-	+-+	-
Sandozol N	+	-	+	-	+	-	+	-	++	-
Solvadine NC Conc.	+	-	+	+	+	-	+	-	-	-
Stablex A	+	-	+	-	+	-	+	-	+++	-
Stablex B	+	-	+	-	+	-	+	-	+	-
Stablex G	+	-	+	-	+	-	+	-	-	-
Sulf. castor oil	+	-	+	+	+	-	+	-	-	-
Surfax W. O.	+	+	+	+	+	+	+	-	++	+
Tergavon C	+	-	+	+	+	-	+	-	+	+
Tergitol Penetrant 7	+	-	+	-	+	-	+	-	+++	+
Tergitol Penetrant 4	+	-	+	-	+	-	+	-	+++	-
Ultrawet E	+	-	+	+	-	-	-	-	-	-
Ultrawet 30 DS	+	-	+	-	+	-	+	-	+	-
Victawet 35 B	-	-	-	-	-	-	-	-	+++	-
Victawet 54 B	-	-	-	-	+	+	-	-	+++	-
Control	+	+	+	+	+	+	+	+	++++	+

Legend:

+ = growth. In Lactobacillus column, indicates degree of growth in comparison to controls.
Inoculum = .05 ml. of 24-hour culture of test organism.
Subculture = .05 ml.
Incubation = 3 minutes, 26° C. 24 hours, 37° C. Lactobacillus, 72 hours, 37° C.
Test organisms = Pseudomonas flourescens, Pseudomonas aeruginosa, Proteus vulgaris (ATC), Proteus (strain D) and Lactobacillus (strain 7).

DISCUSSION

The toxicity of the cationic type surface-active agents has made the practicability of their use as therapeutic agents doubtful, hence the lack of correlation between toxicity and antibacterial activity displayed by Softex AP becomes significant. Despite its lack of local or systemic toxicity in animals under the limitations of the test, it produced definite bactericidal activity against *L. acidophilus* when used in dilutions as great as 1:1,000,000. Triton X-400, Aerosol 1861, and Onyx were superior to Softex AP in bactericidal effect, but they were also much more toxic. The exact composition of Softex AP is un-

known at the present time; should it be pure, undiluted, and chemically reproducible, it may provide material for study in a chemical group which has been theoretically excluded from therapeutic application because of toxicity. Hucker, Brooks, Metcalf, and Von Eseltine[8] have indicated that a high degree of specificity exists in the antibacterial effect of the cationic agents. The results shown in Table II support this finding. It is to be noted that, for purposes of comparison, there is a chemical configuration in the list which corresponds to that of Zephiran Chloride (Onyx and Triton X-400). Further studies of selected cationic agents in relation to the suppression of caries-producing microorganisms is indicated.

Of the nonionic agents, Tween 60 has proved interesting in that it stimulated the growth of *L. acidophilus* when it was used in certain concentrations in liquid cultures. This agent was used by Dubos[9] in his work on a human strain of *Mycobacterium tuberculosis* to produce stimulation of growth in synthetic media.

Work with anionic agents has indicated that substances other than the commonly used alkyl sulfates and alkyl aryl sulfonates may be more effective against bacteria and simultaneously less toxic when administered therapeutically. The literature indicates[10] that the anionic surface-active agents are active against gram-positive organisms only. Results of the present study contradict this finding. Table VII illustrates the effect of the anionic agents against *P. aeruginosa* and two strains of Proteus by the Oxford cup technic. Nutrient agar plates were heavily seeded with the test organisms and 10^{-1} to 10^{-6} dilutions of the test agents were added in sterile penicylinders. The dilutions in which the agents were effective after incubation at 37° C. for 24 hours are noted in Table VII. Table V represents the effect of the agents listed when incorporated in 1:100 concentration in nutrient broth. The broth dilutions were seeded with 0.1 ml. aliquots of a 24-hour subculture of the test organisms and streak plates were made from these tubes after 24 hours' incubation at 37° C. The streak plates were read after 18 hours' incubation at 37° C. and gram stains were made in all cases where growth was present. Table VI illustrates the results of the activity of 10 per cent nutrient broth dilutions of certain anionic agents in 3-minute and 24-hour intervals to distinguish bactericidal from bacteriostatic activity. The inoculum into 10 ml. broth-dilution tubes was 0.05 ml. of a 24-hour broth subculture of *P. aeruginosa, P. fluorescens,* and *P. vulgaris,* and 0.05 ml. subcultures were made into nutrient broth at an interval of 3 minutes and again 24 hours after inoculation. Incubation during the 3-minute period was at 25° C.; during the 24-hour interval it was at 37° C. A resistant strain of *L. acidophilus* was used. The original inoculum was 0.05 ml. of a 24-hour dextrose-broth subculture into 10 ml. tubes of sterile distilled-water dilutions of the agents. Subcultures of either 0.05 or 0.1 ml. were made from the original tube into dextrose broth. Incubation of the Pseudomonas species and Proteus species cultures was 24 hours; the *L. acidophilus* subcultures were incubated for 72 hours. Growth was ascertained in all cases by gram stains from the subculture tubes. It is to be noted that some agents which have

produced local or systemic toxicity when administered by intraperitoneal injection may be nontoxic when administered orally or applied topically. Certain agents which have produced extreme toxicity when used in what might be considered an ordinary concentration, i.e., one per cent, may be found to be nontoxic when administered in the extreme dilutions at which they still produce definite antibacterial effect.

TABLE VII

EFFECT OF ANIONIC AGENTS ON PSEUDOMONAS AND PROTEUS BY THE OXFORD CUP TECHNIC

NAME	PSEUDOMONAS	PROTEUS B	PROTEUS D
Acto 500	(2)		
Ahcowet RS		c17(1)	ca(2)
Aerosol 18	c23	cp32(1)	c27s
Aerosol MA	c17		
Aerosol AY		cp38(1)	c17p19s
Aerosol OT		cp26(1)	c12s
Aerosol 1 B		c15p	c15p19s
Aerosol 22		cap	ca
Aerosol OS		c11s	cap10s++++
Arctic Syntex T		cas	ca
Cerfak F		x	x
Cerfak NS		x	p10s
Detanol P		x	x
Duofol L		p20	p23
Duponol PC		c18	ca
Glyco DS		ca	ca
Glyco TES		ca	ca
Laurel SS		c13p18	p11
Michelene DCA	c20	cap12	cap16
Ninol 737	c16(2)	cp18(2)	(2)
Orvus WA	ca(4)	ca	x
Penetrant 1000		ca	cap11
Petronate	ca(2)	ca(2)	ca(2)
Resolin B	cap16s++	c11s(2)	cp24s(2)
Sandozol N		cap15(1)	p13
Solvadine NC	c17	c14	cp17
Stablex A	ca(2)	x(2)	cp20s(2)
Stablex B		c15	ca
Stablex G		c13	ca
Sulf. castor oil	cas		
Surfax W. O.	(2)		
Tergavon C		s++32	s+15
Tergitol Penetrant 7		ca	ca
Tergitol Penetrant 4	cas	c18	cap11s
Ultrawet E	c16p20s	c13	cp16s
Ultrawet 30 DS.		cap14	cap12
Victawet 35 B		(3)	ca
Victawet 54 B		cap18	ca
Control	x	x	x

Legend:

x = inconclusive evidence.
p = partial inhibition.
c = complete inhibition, measured in millimeters.
a = cup area.
s = stimulation, + indicating degree.

(1) = few colonies in affected area.
(2) = media change.
(3) = surface c20s, deep p26s.
(4) = possible agent change, makes organisms very tenacious in area.

It is to be noted that no attempt was made to use the various classes of surface-active agents at the pH recommended for optimal antibacterial activity. The effect of the introduction of the naphthalene group into the various classes of anionic agents, especially the toxic effect, may be noted in Table I.

SUMMARY

Sixty-nine surface-active agents were injected into mice to ascertain their relative local and systemic toxicity. The effect of these agents upon various microorganisms has been determined, and an attempt was made to correlate the toxicity with antibacterial activity. Data have been presented which indicates that some anionic surface-active agents are effective against gram-negative organisms. Certain general types of surface-active agents have indicated potentialities which merit further study.

REFERENCES

1. Surface Chemistry, American Association of the Advancement of Science, **21**, 1943.
2. Fischer, E. K.: *Soap and Sanitary Chemical* **19**: 25, 1943.
3. Woodard, G., and Calvery, H. O.: Proc. Sc. Sect. Toilet Goods Assoc., June, 1945.
4. Jordan, J. W., Dolce, F. A., and Osborne, E. D.: *J. A. M. A.* **115**: 1001, 1940.
5. Walter, F. E., and Carlson, H. J.: *J. D. Res.* **26**: 353, 1947.
6. Carlson, H. J., and Douglas, H. D.: Unpublished data.
7. Emery, B. E., and Edwards, L. D.: *J. Am. Pharm. A. (Scient. Ed.)* **29**: 254, 1940.
8. Hucker, G. J., Brooks, R. F., Metcalf, D., and Van Eseltine, W.: Food Technology **1**: 321, 1947.
9. Dubos, R. J.: Proc. Soc. Exper. Biol. & Med. **63**: 56, 1946.
10. McCulloch, E. C.: Disinfection and Sterilization, Philadelphia, 1945, Lea & Febiger, p. 363.

DENTAL CARIES IN THE SYRIAN HAMSTER

V. The Effect of Three Different Fluoride Compounds on Caries Activity

PAUL H. KEYES* AND KANWAR L. SHOURIE

From the Division of Dental Research, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y., and the Harvard School of Dental Medicine, Boston, Mass.

THE effect on dental caries in hamsters of three fluoride compounds of different solubility was studied on the hypothesis that the posteruptive caries-inhibitory effect of the fluorides might be related to the availability of the F ion. An attempt was also made to obtain information on a posteruptive systemic effect from fluoride administered parenterally.

EXPERIMENTAL

One hundred two hamsters were raised on Purina Rabbit Checkers and tap water with fresh vegetable supplements. Forty-eight male and fifty-four female animals, 30 to 40 days old, were distributed as equally as possible into 6 groups (Table II).

A ration which contained 30 per cent whole powdered milk, 30 per cent whole wheat flour, 20 per cent corn starch, 15 per cent confectioners' sugar, 4 per cent alfalfa, and 1 per cent NaCl was available to all groups ad libitum. Supplements of fresh vegetables were given once a week.

Group I received the experimental ration and distilled water.

Group II received the experimental ration into which NaF, solubility 4.045 Gm./100 c.c. H_2O,[1, 2] was thoroughly incorporated at a level of 50 ppm F.

Group III received the experimental ration. The sodium fluoride content of the drinking water was adjusted so that the intake of fluoride from this source would approximate that ingested in the diet fed to Group II. The average food consumption of males and females in Group II was determined for nine periods and the intake of fluorine was calculated for each period. After each fluoride estimation the water concentration of F was adjusted so that the average intake from the water for the preceding period would approximate that which had been consumed in the food during the past period. During the first period the water level of F was arbitrarily adjusted to 50 ppm.

It was recognized that by restricting rations and water, the F intake could have been more accurately regulated. Space and facilities were not available to permit such careful control, and in view of the information desired, highly refined technics were not considered necessary. As the food and water intake of hamsters tended to be very close to a 1:1 ratio under the experimental conditions described, 50 ppm F in both food and water during the entire experimental period probably would have been satisfactory.

Read at the 26th General Meeting of the International Association for Dental Research. Meeting at Rochester, N. Y., on June 18-20, 1948 (J. D. Res. 27: 748, 1948).

Received for publication August 14, 1948.

*Now at the Harvard School of Dental Medicine.

Group IV animals received NaF subcutaneously in an amount approximating that consumed by Group II. It was necessary to use a 1 per cent aqueous solution of NaF since both a peanut oil-beeswax and gelatin suspension caused severe irritation of the skin. Although it soon became apparent that there was an unavoidable bias on these animals, they were continued on subcutaneous injections 3 times a week of 0.2 c.c. of a 1 per cent NaF solution until the end of the experimental period.

Group V received the experimental ration into which CaF_2, solubility 0.004 Gm./100 c.c. H_2O,[1, 2] was carefully incorporated at a level of 50 ppm F.

Group VI received 50 ppm F as Na_2SiF_6 thoroughly mixed in the experimental ration. The solubility of sodium fluorosilicate is 0.762 Gm./100 c.c. H_2O.[1, 2]

Daily food and water consumption was measured for the groups. Weights were taken at weekly intervals. Males were maintained on the experimental regimen for 83 days. Because females are less susceptible to dental caries than males,[3] they were kept on the experimental regimen for 106 days. Two male animals died, one in Group I and one in Group III.

Heads were fixed in formalin, and jaws were examined under 14× magnification. Findings were charted and scored by methods previously described[4] by two observers with closely agreeing results.

RESULTS

There was no reduction in food and water consumption in the groups that received fluorides and no inhibition of growth (Table I). The effect of the fluorides on caries activity is reported in Tables II and III.

Group I (Control).—All animals had a high incidence of caries with the average male score (160.5) exceeding the average female score (126.8). All molar teeth in the male group were affected to some extent and 99 per cent of female molars were involved. In both sexes upper and lower second and third molars showed the greatest amount of destruction; the third molars were particularly susceptible (Table III).

Group II (50 ppm F as NaF in food).—There was a striking reduction in caries activity in these animals. Average male score was 0.84 with three animals with no gross lesions. The average female score was 6.2 with four animals free from gross lesions. The greatest reduction in caries activity appeared to take place in third molars.

Group III (NaF in drinking water).—The reduction of caries activity in this group was as effective as in Group II; average male score was 0.6; average female score, 6.9. As in Group II the most striking reduction in caries activity occurred in third molars.

Group IV (NaF subcutaneously).—The reduction in caries activity in this group (Table II) cannot be attributed solely to the systemic effect of fluoride. Hamsters may contaminate their food and cages with urine and feces. Coprophagy is common, and the animals habitually clean themselves off after urination. These circumstances result in contamination of the oral cavity with fluoride compounds, and thus a local action of fluoride is possible.

TABLE I

AVERAGE INITIAL WEIGHT, WEIGHT GAINED, AND FINAL WEIGHT

DAYS ON EXPERIMENT	GROUP I CONTROL		GROUP II NaF (FOOD)		GROUP III NaF (H_2O)		GROUP IV NaF (S.Q.)		GROUP V CaF_2 (FOOD)		GROUP VI Na_2SiF_6 (FOOD)	
	M	F	M	F	M	F	M	F	M	F	M	F
0	57	58	57	61	55	60	54	57	60	58	64	55
5	9	9	11	10	8	10	8	10	8	11	9	10
12	8	10	5	8	7	11	7	10	11	11	10	10
19	4	5	7	5	3	10	−2	4	6	9	7	9
26	5	7	4	9	4	4	2	2	0	2	2	0
33	4	4	3	4	7	8	2	4	5	7	2	9
40	1	3	0	7	2	7	0	−3	3	7	3	9
47	1	6	4	7	4	7	5	6	3	5	3	3
55	4	3	3	3	3	4	2	2	2	5	3	6
61	3	2	2	4	3	2	2	1	3	4	1	2
68	2	2	1	3	1	3	6	13	3	3	4	6
75	3	1	2	3	4	1	3	2	1	1	3	1
83		5		2		2		6		6		5
90		−1		3		3		6		−1		0
96		2		6		1		5		−1		2
106												
Totals	102	118	102	133	107	133	94	129	107	130	114	126

TABLE II

LITTER DISTRIBUTION OF CARIES FINDINGS

LITTER	GROUP I CONTROL		GROUP II NaF (FOOD)		GROUP III NaF (H_2O)		GROUP IV NaF (S.Q.)		GROUP V CaF_2 (FOOD)		GROUP VI Na_2SiF_6 (FOOD)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	180	281	4.0	3.25	0.0							
2						10.0	85.0	120.0	185	142.0		
4	73	103	0.0	0.0	0.25	0.0	3.75					1.0
5	189		0.5		0.0		56.5	1.75		51.5		2.0
6	140	130	0.0	0.25	3.25	0.75	34.0	51.25		57.5	56.25	1.0
7		155		5.0		9.25		14.25		21.5		
8	220	148	0.5	0.0	+							1.5
9						9.75	62.25	11.5	230	139.0	70.25	30.25
10		84		47.0		7.0		25.5	176	96.0	78.5	
11	206	89	1.0	0.25	0.25	0.0	43.0	9.0				0.5
12	+		0.0						154	80.0	2.0	
13	116	53	0.75	0.0	0.5		79.0		199		29.25	12.0
14						25.5		29.75	126		56.75	
15		98		0.0	0.25	0.0			189	46.0	15.75	0.0
16								0.0		86.5		3.0
18							13.75		226		5.5	
Ave. score	160.5	126.8	0.84	6.2	0.6	6.9	47.2	29.2	185.6	80.0	39.3	5.7
Ave. molars affected	12.0	11.9	1.3	1.9	1.0	1.9	9.8	8.8	12.0	11.9	7.0	3.0
Ave. no. of cavities	15.4	16.3	1.3	1.9	1.0	1.9	13.0	10.8	14.7	19.0	7.7	3.0
Teeth with ?? caries (Ave.)	0.0	0.0	3.3	3.6	4.5	3.4	1.2	0.8	0.0	0.1	3.1	3.9

Group V (50 ppm F as CaF_2 in food).—There was no reduction in caries activity in male animals, score 185.6. In females the extent of cavitation, represented by an average score of 80.0, was slightly reduced in comparison with control females; however, the females on CaF_2 had as many teeth affected and as great a number of cavities as the control animals. These data may indicate

TABLE III

AVERAGE PERCENTAGES OF MOLAR CROWNS DECAYED

	MAXILLARY			MANDIBULAR		
	1ST	2ND	3RD	1ST	2ND	3RD
Males						
I Control	25.4	72.9	96.0	31.8	52.3	76.7
II NaF (food)	0.0	0.0	1.1	0.06	0.8	0.0
III NaF (H_2O)	0.0	0.0	0.0	0.0	1.3	0.0
IV NaF (s.q.)	7.8	23.9	38.7	8.7	20.4	7.4
V CaF_2 (food)	33.1	80.4	97.5	55.7	71.3	68.7
VI Na_2SiF_6 (food)	7.3	0.2	7.5	14.0	45.8	6.4
Females						
I Control	37.0	64.1	76.2	28.9	44.4	27.7
II NaF (food)	0.4	3.4	0.0	0.01	8.8	0.0
III NaF (H_2O)	0.0	1.6	0.0	0.0	12.8	0.0
IV NaF (s.q.)	4.9	15.2	17.2	4.3	17.8	5.0
V CaF_2 (food)	15.3	47.9	61.8	16.6	23.2	15.2
VI Na_2SiF_6 (food)	0.9	0.06	0.9	1.5	7.7	0.9

a slight inhibitory effect of CaF_2. Unfortunately, sufficient litter-mate comparisons are not available to determine whether the slightly smaller female scores are statistically significant.

Group VI (50 ppm F as Na_2SiF_6 in food).—Male and female animals in this group showed a definite reduction in caries activity with respective scores of 39.3 and 5.7, but the reduction was not so pronounced as that in Groups II and III when a comparison is made of scores, molars affected, and the number of cavities.

DISCUSSION

The inhibition of caries activity in hamsters by NaF has been demonstrated in other experiments.[5, 6] In this study, when NaF was added to the ration or to a calcium-free drinking water, caries inhibition was marked. Since sodium fluorosilicate was moderately effective in reducing caries activity and calcium fluoride was essentially ineffective, it appears that the local posteruptive inhibition of caries activity by fluoride is related to the quantity of F ions present. The source of the ions and the means of their ingestion, either in food or water, apparently is of little importance, providing the ions are in such form as to be available.

In studying certain effects of fluorides, results from the artificial incorporation of fluorides into rations may not be comparable to the action of natural fluorides found in foodstuffs. Until additional information is available, the biological activity of natural food fluorides might be considered relative to the digestibility of the food, the solubility of the fluoride compound, the proximity and quantity of calcium compounds in the diet, etc. The effect of fluorides in water supplies may be affected by the calcium content of the diet. In view of our findings, reservations seem appropriate in evaluating the rational and the therapeutic value of compounds containing highly insoluble fluorides for the control of dental caries.

Smith and Leverton[7] found great differences in toxicity of different fluoride compounds, which were apparently related to differences in solubilities of the

compounds. It required more cryolite and calcium fluoride than other soluble fluorides to produce severe changes in the teeth; however, all the compounds of fluorine studied produced depigmentation of the incisors when incorporated at a 14 ppm level. Apparently the amount of fluorine required to cause initial changes in rat incisors was so small that differences in solubility of compounds were not a factor.

No consistent changes or variations in pigmentation could be observed in incisors of hamsters fed 50 ppm fluorine in the three fluorine compounds tested. The incisor enamel of those animals which received the subcutaneous injections of fluoride showed intermittent pitted lines characteristic of enamel hypoplasia. Apparently there is a species difference in the susceptibility of animals to fluoride toxicity as judged by pigmentary changes in the enamel.

Small laboratory animals do not appear well suited for studying the systemic effect of fluorides on erupted teeth. When fluorides are given by stomach tube or by parenteral routes, they will get into the oral cavity through coprophagy and urine contamination. It is extremely doubtful that specially designed cages could completely eliminate this source of bias in hamsters, and probably not in other small laboratory animals. If one assumes that the inhibition of caries was due to a systemic process, the mechanism would appear to be not so efficient as the local effect on teeth. Group II and III males ingested about 5 mg. of F per week orally and Group IV males received about 6 mg. of F parenterally. The respective scores for Groups II, III, and IV males are 0.84, 0.6, and 47.2.

Some evidence of a pre-eruptive systemic effect may be gathered from the observation that the greatest reduction took place in the third molar region. These teeth were either in the late stages of calcification or early eruption at the time the experiment was started. The color of these teeth was definitely whiter, and they appeared harder than the first and second molars. There was no evidence of mottling. Cox, Matuschak, Dixon, Dodds, and Walker,[8] Cheyne,[9] and Norvold and Armstrong[10] have observed reductions of caries activity in rats following the pre-eruptive administration of fluoride. The remarkable reduction in third molars of animals in Groups II and III may be the result of a combined pre-eruptive and posteruptive effect.

SUMMARY AND CONCLUSIONS

Under the conditions of this experiment 50 ppm of fluorine, as sodium fluoride added to food and water, was very effective in reducing caries activity in the molar teeth of hamsters; sodium fluorosilicate was somewhat less effective, and calcium fluoride was essentially ineffective.

Hamsters do not appear well suited for studying the posteruptive systemic pathways of fluoride action on caries activity due to contamination of the oral cavity with urine and feces.

In dealing with certain biological effects of fluorine, it seems desirable to consider the species under examination and the source and availability of the F ions.

REFERENCES

1. Carter, R. H.: Solubilities of Some Inorganic Fluorides in Water at 25° C., *Indust. & Engin. Chem.* **20**: 1195, 1928.
2. Carter, R. H.: Solubilities of Fluorosilicates in Water, *Indust. & Engin. Chem.* **22**: 886, 1930.
3. Keyes, P. H.: Dental Caries in the Syrian Hamster. IV. The Effect of Gonadectomy on Caries Activity and Body Growth, *New York J. Dent.* **18**: 172, 1948.
4. Keyes, P. H.: A Method of Recording and Scoring Gross Carious Lesions in the Molar Teeth of Syrian Hamsters, *J. D. Res.* **23**: 439, 1944.
5. Dale, P. P., Lazansky, J. P., and Keyes, P. H.: Production and Inhibition of Dental Caries in Syrian Hamsters, *J. D. Res.* **23**: 445, 1944.
6. Dale, P. P., and Keyes, P. H.: Inhibition of Experimental Dental Caries in Syrian Hamsters by Fluorine and Iodoacetic Acid, *J. D. Res.* **24**: 194, 1945.
7. Smith, M. C., and Leverton, R. M.: Comparative Toxicity of Fluorine Compounds, *Indust. & Engin. Chem.* **26**: 791, 1934.
8. Cox, G. H., Matuschak, M. C., Dixon, S. F., Dodds, M. L., and Walker, W. E.: Experimental Dental Caries. IV. Fluorine and Its Relation to Dental Caries, *J. D. Res.* **18**: 481, 1939.
9. Cheyne, V. D.: An Experimental Approach to Some Dental Problems by Use of a Procedure to Salivary Gland Extirpation in Rats, Thesis, University of Rochester, 1940.
10. Norvold, R. W., and Armstrong, W. D.: Mechanism of Fluorine Inhibition of Caries in the Rat, *J. D. Res.* **22**: 243, 1943.

THE INFLUENCE OF THE BLOOD CALCIUM LEVEL UPON THE ACTION OF FLUORINE IN PREDENTIN AND DENTIN FORMATION*

J. T. IRVING, PH.D., M.D.†

Department of Physiology, Medical School, University of Cape Town, Union of South Africa

IN previous papers[5, 13] it has been shown that when NaF is injected into rats, it affects the developing predentin in such a way that when calcification occurs, a typical pattern of structure can be demonstrated histologically. In instances of normal rats and those with high Ca rickets, the effect on the predentin cannot be seen by histological staining and is not evident by such methods until this tissue calcifies. In the case of animals with low Ca rickets, on the other hand, a fine calcified line stainable by hematoxylin is seen from about twelve hours onward and this finally blends with the spontaneous calcification in a way which is different from that in normal animals.[8] Irving[5] showed by measurement that this fine line corresponded to the experimental dentin line of Irving and Weinmann.[10] More details of these histological changes will be found in the papers quoted.

However, attempts to explain why the experimental dentin line was prematurely calcified only in the predentin of animals with low Ca rickets were not particularly successful. Irving and Nienaber[9] investigated the serum Ca level of rats with the two types of rickets and the effect of F upon this. In high Ca rickets the blood level Ca was normal, in low Ca rickets it was low. The effect of F was the same in both cases, namely, to cause an immediate fall in the serum Ca level. No other specific effects were noted.

Since the only difference between the two groups of animals lay in the original levels of blood Ca and blood inorganic P, it was decided to investigate whether the calification of the experimental dentin line could not be altered by procedures known to affect the blood Ca level. Starvation is known to lower the serum Ca of rats with high Ca rickets,[2, 11, 15] and vitamin D will cause a transient rise in the blood Ca of rats with low Ca rickets.[7] It was considered that some elucidation of the problem might be effected by altering the level of blood Ca by these two procedures in the two groups of rats, and then testing the effect of F, to see whether the differences in the calcification of the line were bound up with the level of blood Ca. The results to be reported showed that this was so.

EXPERIMENTAL

Diets.—The diets employed were the same as those previously used,[5] and are designated by the same numbers. Diet 2 was the rachitogenic diet of Steenbock and Black[14] and had an average Ca:P ratio of 4.5. Diet 3 was a low Ca diet, with a Ca:P ratio of 0.29.

Rats.—The animals used were Wistar Institute strain albino rats, kept in a special animal house at a temperature of 68° to 70°. Males and females were used indiscriminately, the results found being the same in both sexes.

Received for publication June 16, 1948.

*The expenses of this work were defrayed by grants from the Staff Research Fund, University of Cape Town, and the South African Council for Scientific and Industrial Research.

†Fellow of the University of Cape Town.

Experimental Procedures:

Diet 2.—Rats were transferred to this diet when they weighed from 50 to 60 grams, and they were maintained on it for 28 days by which time they had well-marked rickets. Their daily intake of diet was then restricted for 1, 1⅓, 1¾, 2, or 3 days, so that they lost about 3 grams weight per day; they were allowed unlimited access to water. At the end of this time, they were injected subcutaneously with a 2 per cent NaF solution, at a dose level of from 6 to 7 mg. F/kg. body weight, and were then returned to Diet 2. They were then killed from 1 to 4 days after the F injection and their teeth were examined histologically. Some control animals had their dietary intake restricted and were then killed; others were given NaF without dietary restriction. Three litters of rats were treated in this way in accordance with the following timetable (Table I).

TABLE I

RAT NUMBER	DAYS OF DIETARY RESTRICTION	DAYS OF SURVIVAL AFTER NaF INJECTION
1146	1	Not injected
1147		1
1148		2
1149		3
1150		4
1151	1⅓	1⅔
1152		2⅔
1153		3⅔
1154		4⅔
1180	1¾	1
1181		2
1182		3
1179	2	Not injected
1183, 1187		1
1184, 1188		2
1185, 1189		3
1186	3	Not injected
1191		1
1192		2
1193		3
1194		4
337	Not restricted	1½
338		2

Since it was important to know the blood Ca level at the time of F injection, rats from 3 further litters were treated as follows:* After 28 days on Diet 2, the animals were divided into 4 groups. One group was continued on the diet for 2 days, and the other 3 groups had their dietary intake restricted for 2 days. All rats but one or two of each group were then killed, their bloods pooled and examined for serum Ca. The remaining rat or rats were injected with NaF solution and killed after 2 further days for histological examination. The following timetable (Table II) was employed.

Diet 3.—The rats on this diet were treated, in the preliminary stages, in the same way as those on Diet 2. When they had attained a weight of from 50 to 60 grams, they were transferred to Diet 3 and maintained on it for 28 days. The experimental animals were then given one dose of 27 I.U. of vitamin D

*The experiments with both Diets 2 and 3 involving blood Ca estimations were carried out in the Department of Physiology, University of Chicago. The writer is indebted to Dr. Franklin McLean for his hospitality, to Dr. Z. Miller for carrying out the Ca estimations, and to Dr. J. Waldman for bleeding the rats. The method of Ca estimations employed was that of Kramer and Tisdall[12] as modified by Clark and Collip.[3]

TABLE II

GROUP	DAYS OF DIETARY RESTRICTION	NO. OF RATS KILLED FOR SERUM Ca ESTIMATION	SERUM Ca	NO. OF RATS INJECTED WITH NaF (KILLED 2 DAYS LATER)
A	Nil	6	9.89	2
B	2	3	5.90	1
C	2	3	4.92	1
D	2	6	6.84	2

by mouth. Two days later they were injected subcutaneously with 2 per cent NaF solution at a dose level of about 9 mg. F/kg. body weight. They were then killed at intervals of from 2 to 8 days after the F injection. Some control animals were given vitamin D only, while others were only injected with F. Table III gives the timetable adopted.

TABLE III

RAT NUMBER	DAYS OF SURVIVAL AFTER VITAMIN D ADMINISTRATION	DAYS OF SURVIVAL AFTER NaF INJECTION
1065, 1112, 1113 1143, 1156	4	2
1066, 1115, 1116 1158	6	4
1144	7	5
1068, 1118, 1160	8	6
1145, 1162	10	8
1111, 1142[x]	Not dosed	Not injected
1067	6	
1069	8	
1114, 1155[o]	Not dosed	2
1117, 1157[o]		4
1119, 1159[o]		6
1161[o]		8

[x]Killed after 28 days on Diet 3.
[o]Injected after 30 days on Diet 3.

To obtain information of the level of the blood Ca at the time of the F injection, the following experiments were carried out on 3 further litters. These rats were given, as before, Diet 3 for 28 days after attaining a weight of 50 to 60 grams. They were then divided into 4 groups. The rats of 2 groups were given 30 I.U. of vitamin D by mouth in cod-liver oil, and the rats of the other two groups were given an equal volume of corn oil. After 2 days, all but one rat of each group were killed, and the pooled bloods were estimated for serum Ca. The remaining rat of each group was injected with NaF solution and allowed to live for 2 further days. These rats were then killed and their teeth examined histologically. Table IV shows the timetable used.

TABLE IV

GROUP	DOSE 2 DAYS BEFORE DEATH WITH	NO. OF RATS KILLED FOR SERUM Ca ESTIMATION	SERUM Ca VALUES	NO. OF RATS INJECTED WITH NaF (KILLED 2 DAYS LATER)
A	Oil	7	6.30	1
B	Oil	3	6.52	1
C	Vitamin D	9	9.10	1
D	Vitamin D	3	10.35	1

Histological examination.—The skulls were fixed in formol-saline and decalcified in nitric acid-formol-saline. The lower jaws were removed, and, after embedding in paraffin, the upper incisor teeth were cut semiserially in longitudinal section. They were stained with hematoxylin and eosin, and in some cases, by the method of silver impregnation (Gömöri[4]). The changes in the labial dentin and predentin, where they are most clearly seen, were examined qualitatively and in some cases measured, those on the lingual side also being noted.

RESULTS

Rats with high Ca rickets.—Many of the animals had incipient tetany; some showed violent tetanic attacks, after dietary restriction, which were accentuated by the injection of NaF. This was most noticeable in the animals starved for 1¾ and 2 days, and one animal in each of these groups died after the NaF injection.

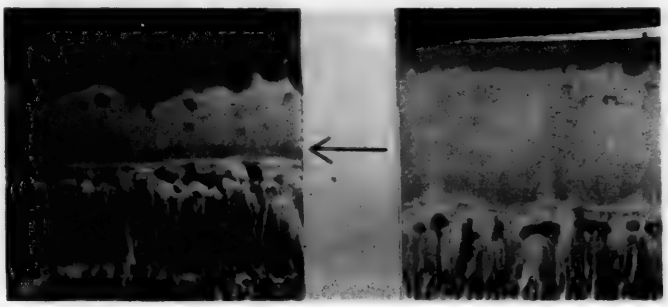


Fig. 1. Fig. 2.

The microphotographs are of the labial incisal dentin and predentin, and, with the exception of Fig. 3, near the formative end of the tooth. Orig. Mag. ×200.

Fig. 1.—Rat 1184, which had been on Diet 2 for 28 days and had its dietary intake restricted for 2 days. It had then been injected with NaF solution, and killed after a further 2 days. Arrow points to calcified experimental dentin line in predentin. Other fine line, nearer odontoblasts, is border between early and late predentin. Compare with Fig. 4.

Fig. 2.—Rat 337, which had been on Diet 2 for 28 days but had not been starved. It had then been injected with NaF solution and killed 1½ days later. No calcified experimental dentin line occurs in predentin. Separation of dentin and organic enamel is an artefact.

Examination of the teeth showed the presence of a fine calcified line in the predentin of all the rats which had been restricted for 1¾ days or longer and then injected with NaF, and in 2 rats restricted for 1⅓ days. None of the rats restricted for 1 day showed it. This line appeared identical with the experimental dentin line previously found in the teeth of animals with low Ca rickets injected with NaF. Histologically it had the same characteristics, both with respect to hematoxylin and eosin staining (Fig. 1) and also to silver impregnation, when it appears as a narrow argyrophobic layer.[8] The line was not demonstrable by either method of staining in animals which had been injected without previous dietary restriction (Fig. 2), or which had been restricted but not injected.

The distances of the line from the pulpal edge of the predentin were measured and plotted against the time in days after the injection of NaF. The points fell on to a straight line, which cut the origin when extrapolated. This

showed that, as in the case of animals with low Ca rickets, injected with NaF, the line was laid down at the time of the NaF injection and was the experimental dentin line. The line moved outward with the incremental growth of the predentin, which in the present case averaged 6 μ per day. This low figure would be expected in animals with high Ca rickets.

The serum Ca figures quoted in Table II show that at the time of injection, the blood Ca had been lowered by dietary restriction to a level equal to that of rats with low Ca rickets (Table IV).

In four of the restricted rats which had lived for 3 or more days after NaF injection, calcification had proceeded sufficiently far at the incisal end of the tooth for the experimental dentin line to blend with this. The reaction usually found in high Ca rickets was present, this line being preceded by a hypocalcified layer which was separated from the pre-experimental dentin by a calciotraumatic line (Fig. 3).

Fig. 3.—Rat 1194, which had been on Diet 2 for 28 days and then had its dietary intake restricted for 3 days. It was then injected with NaF solution and killed after a further 4 days. Arrow points to experimental dentin line, *a*—hypocalcified dentin, separated from pre-experimental dentin by calciotraumatic line, *b*—normal predentin. Section is from incisal part of tooth.

Rats with low Ca rickets.—Several of the control rats had tetany after the NaF injection; this happened with none of those previously dosed with vitamin D. One litter was given rather more NaF than usual; all the rats of this litter getting NaF alone died while all those previously given vitamin D survived.

Examination of the teeth showed that the animals given NaF only had the usual fine calcified line in the predentin (Fig. 4). Those given vitamin D only had the usual healing reaction in the teeth caused by this substance[6] (Fig. 5). Seventeen rats were given vitamin D followed 2 days later by the injection of NaF. The teeth of those killed early in the experiment did not show any calcified experimental dentin line in the predentin. Later on, however, when the predentin formed at the time of the injection calcified, the same F reaction was seen as occurs in rats with low Ca rickets.[8] Thus the premature calcification of the experimental dentin line had been prevented by the vitamin D, but the predentin had been invisibly altered by the F, so that when calcification occurred, the line also calcified and was revealed.

The figures in Table IV show that at the time of the injection, 2 days after vitamin D administration, the blood Ca figures of the dosed animals had been raised considerably above those of the controls. The administration of corn oil was apparently without effect upon the blood Ca level. This observation con-

firms that of Booth, Henry, and Kon,[1] that fat has no antirachitic effect when given to animals on low Ca high P diets.

DISCUSSION

It had previously been suggested (Irving[5]) that the premature calcification of the experimental dentin line in animals with low Ca rickets and the fact that this does not happen in normal rats or those with high Ca rickets might be connected with the level of the blood Ca. The present results support this contention. While it is possible that other metabolic changes induced by the procedures adopted (for example, in the blood inorganic P level) might be bound up with these findings, the part played by Ca in calcification and the known affinity between this element and F make it seem most probable that the level of the blood Ca is the deciding factor as to whether the odontoblasts lay down a line in the predentin which calcifies early or not.

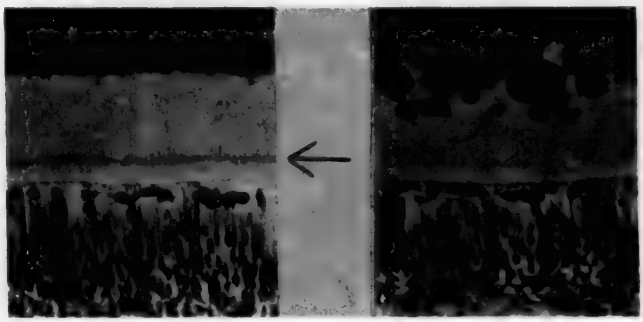


Fig. 4. Fig. 5.

Fig. 4.—Rat 1155, which had been on Diet 3 for 30 days and was then injected with NaF solution. It was killed 2 days later. Arrow points to calcified experimental dentin line in predentin.

Fig. 5.—Rat 1156, which had been on Diet 3 for 28 days and was then given 27 I.U. of vitamin D by mouth. Two days later it was injected with NaF solution and killed after a further 2 days. No experimental dentin line is seen in predentin. Only change is formation of interglobular dentin which is early healing reaction caused by vitamin D.

It can thus be concluded that under all circumstances, F affects the matrix as laid down, so that it ultimately calcifies in a specific way. When the blood Ca is normal, the change in the matrix is not demonstrable by histological means till calcification of the dentin is about to occur. If the blood Ca is low, the experimental dentin line calcifies prematurely and stains with hematoxylin. It should be noted that irrespective of whether or not this line calcifies prematurely, it still takes its place in the sequence of events of the F calcification reaction when the predentin calcifies.

It has been shown that two fluorine reactions can be obtained in calcifying dentin. One, the classical appearance described by Schour and Smith, in normal animals and those with high Ca rickets, and another, differing in some respects, in animals with low Ca rickets.[8] The present results show that these phenomena are still specifically obtained in the various groups of animals in spite of the transient alteration of the blood Ca before F is given. In other investigations carried out in this department, it has been invariably found that the blood Ca very soon returns to its original level when starved animals with

high Ca rickets are re-fed on Diet 2; and that after 6 days, it has subsided to its original low level in animals with low Ca rickets after the vitamin D dosage used in the present experiments.[7] The calcifying dentin does not incorporate the experimental dentin line, or the site of this line in rachitic animals, till at least 6 days have elapsed, by which time the blood Ca level has returned to its original value. This is probably the reason why the F reaction in the dentin is still specific for the type of rickets from which the animal is suffering. This question merits further investigation.

SUMMARY

1. The experimental dentin line is a hypercalcified layer which forms the central part of the calcification response of the dentin to the action of fluorine. It is laid down in the predentin which has formed at the time of the injection of F.

2. The results of the experiments reported in this paper show that if the blood Ca is low at the time of the injection of F, the experimental dentin line calcifies prematurely, shortly after the F has been given. If the blood Ca is normal, the predentin forming at the time is affected by the F but does not become hypercalcified till spontaneous calcification occurs.

REFERENCES

1. Booth, R. G., Henry, K. M., and Kon, S. K.: A Study of the Antirachitic Effect of Fat on Rats Receiving High Calcium—Low Phosphorus Rachitogenic Diets, *Biochem. J.* **36**: 445, 1942.
2. Cavins, A. W.: The Effect of Fasting (and Feeding) on the Calcium and Inorganic Phosphorus in Blood Serums of Normal and Rachitic Rats, *J. Biol. Chem.* **59**: 237, 1924.
3. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification, *J. Biol. Chem.* **63**: 461, 1925.
4. Gömöri, G.: Silver Impregnation of Reticulum in Paraffin Sections, *Am. J. Path.* **13**: 993, 1937.
5. Irving, J. T.: The Action of Sodium Fluoride on the Dentin and Predentin of the Incisor Teeth of Rats Consuming Diets Containing Calcium and Phosphorus in Various Ratios, *J. D. Res.* **22**: 447, 1943.
6. Irving, J. T.: The Action of Vitamin D Upon the Incisor Teeth of Rats Consuming Diets With a High or Low Ca:P Ratio, *J. Physiol.* **103**: 9, 1944.
7. Irving, J. T.: The Effect of Dietary Restriction Upon the Incisor Teeth of Rachitic Rats, *J. Physiol.* **105**: 16, 1946.
8. Irving, J. T.: The Action of Fluorine Upon the Calcification of the Dentin in Rats With Low Calcium Rickets, *J. D. Res.* **28**: 17, 1949.
9. Irving, J. T., and Nienaber, M. W. P.: The Effect of Sodium Fluoride on the Teeth and Blood Calcium and Inorganic Phosphorus Levels of Rachitic Rats, *J. D. Res.* **25**: 327, 1946.
10. Irving, J. T., and Weinmann, J. P.: Experimental Studies in Calcification. VI. Response of Dentin to Injections of Strontium in Albino Rats, *J. D. Res.* **27**: 669, 1948.
11. Kramer, B., Shear, M. J., and Siegel, J.: Composition of Bone. X. Mechanism of Healing in Low Phosphorus Rickets, *J. Biol. Chem.* **91**: 271, 1931.
12. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J. Biol. Chem.* **47**: 475, 1921.
13. Schour, I., and Smith, M. C.: Injections of Sodium Fluoride on Enamel and Dentin of the Incisor of the Rat, *Proc. Soc. Exper. Biol. & Med.* **32**: 1, 1934.
Idem: The Histological Changes in the Enamel and Dentin of the Rat Incisors in Acute and Chronic Experimental Fluorosis, *U. of Ariz. Col. Agric. Tech. Bull.* **52**: 69, 1934.
14. Steenbock, H., and Black, A.: Fat Soluble Vitamins. XXIII. The Induction of Growth-promoting and Calcifying Properties in Fats and Their Unsaponifiable Constituents by Exposure to Light, *J. Biol. Chem.* **64**: 263, 1925.
15. Wilder, T. S.: The Tetany of Fasting in Experimental Rickets, *J. Biol. Chem.* **81**: 65, 1929.

INHIBITORY EFFECT OF FLUORIDE ON TOOTH DECALCIFICATION BY CITRATE AND LACTATE IN VIVO

I. ZIPKIN AND F. J. McCLURE

National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

INTRODUCTION

WITHIN recent years, decalcification of molar teeth in vivo has been produced experimentally in a number of laboratory animals.[1-7] Extensive loss of molar dentin and enamel in vivo was first reported in rats given dilute acids and acid drinks and beverages in place of drinking water.[1] Similar results have been noted in hamsters, monkeys, and puppies.[2, 3, 4] The low pH of these acid solutions in most cases accounted for the observed tooth destruction.

The presence of citrate in many soft drinks and fruit juices[8] suggested in addition a specific decalcifying effect of the citrate ion, in keeping with its known solubilizing effect on insoluble calcium salts.[9-12] As was anticipated, therefore, citrate solutions caused a significant decalcification of rats' molar tooth surfaces, not duplicated by lactate solutions at the same nearly neutral pH.[6]

Recently, Wynn and Haldi[5] observed the decalcifying effects of a number of fruit and vegetable juices on rats' molar tooth surfaces in vivo, but were not able to correlate consistently the pH or the amount of citrate presumed to be present in the fluids with the amount of tooth destruction. Their results seemed to lend additional support to the suggestion that other anions in addition to citrate may decalcify tooth surfaces in vivo.[6, 13, 14]

The role of fluorine in inhibiting tooth decalcification in vivo has been explored thus far most extensively by Restarski, Gortner, and McCay,[2] Gortner, Restarski, Bieri, and McCay,[3] and Bieri, McCay, Restarski, and Gortner.[4] Their results, in general, demonstrate a significant inhibitory effect of fluoride on the tooth decalcifying action of phosphoric, sulfuric, lactic, and citric acids, and of sucrose-phosphoric acid mixtures. As little as 1.0 ppm fluorine appeared to be inhibitory, but the degree of protection afforded did not seem to vary directly with the amount of fluoride incorporated in the test solutions.[3]

It was the purpose of this study, therefore, to obtain additional evidence regarding the effect of fluoride on acid decalcification produced particularly by lactic acid, and to ascertain also the effect of fluoride on nonacid decalcification brought about by the citrate ion. Citrate solutions of pH 6.3 to 6.5 were used in this study. Although these solutions are slightly acidic, the term "non-acid" refers to the effect of the citrate anion, since the hydrogen ion is practically ineffective in producing loss of tooth substance at this pH. The effect of intraperitoneally injected fluoride on acid and nonacid decalcification was also studied in an attempt to evaluate the significance of the oral surface effects of fluoride.

Presented in part at the Washington, D. C., Section of the International Association for Dental Research, May 19, 1948.

Received for publication September 19, 1948.

PLAN OF THE EXPERIMENT

The general plan of the study is indicated in Tables I and II. Weanling rats of approximately 21 days of age were divided into tetrads composed of litter mates. The study was divided into Experiments A, B, C, and D, and each experiment consisted of a preliminary period of 28 days and a test period of variable length. Before giving the tooth substance-removing fluids, the respective fluoride solutions alone were offered ad libitum during the 28-day preliminary period in an attempt to "fluorinate" the teeth. The Control and "F Inject" groups received distilled water during this period, and the F Inject groups were injected intraperitoneally with 0.5 mg. fluorine daily (1.0 ml. of a sodium fluoride solution containing 500 ppm fluorine).

Following the preliminary period, the test solutions containing added fluoride were administered as the sole source of fluid, as indicated in Tables I and II. The Control and F Inject groups received the test solutions without added fluoride, and the F Inject groups received 0.5 mg. of fluorine per day injected intraperitoneally. The diet, consumed ad libitum, was made up as follows: cornstarch, 66.5 per cent; whole milk powder, 27 per cent; yeast, 5 per cent; cod liver oil, 1 per cent, and sodium chloride (containing added traces of iron and copper), 0.5 per cent. This diet was adequate for normal growth in rats.

At the end of the test period, the rats were sacrificed and the heads were autoclaved to facilitate removal of the jaws for examination. The molar teeth were scored according to the method proposed by Restarski, Gortner, and McCay.[15] The average score for the teeth of each quadrant per rat is presented in Tables I and II.

The data were submitted to statistical analysis. Inasmuch as Experiments A and B are essentially similar, the data were combined and submitted to analysis of variance[16] as shown in Table III. In this treatment, the data for the 50 ppm and the F Inject groups were omitted. The former data were not included since it is apparent that 50 ppm fluorine affords no further protection against erosion than 5 ppm fluorine. The data for the F Inject group were omitted since they represent systemically acquired fluorine as compared to orally administered fluorine. The data of Experiment C were analyzed by applying Fisher's "t" test,* to determine the significance of the difference between the means. The data of Experiment D were studied by means of the analysis of variance, and the results are expressed in Table IV.

---

*Fisher's "t" test $= \frac{\text{Difference between the means}}{\text{Standard error of difference between the means.}}$ Standard error of difference between the means $= \sqrt{(\text{S.E.})_1^2 + (\text{S.E.})_2^2}$, where $(\text{S.E.})_1$ and $(\text{S.E.})_2$ = standard errors of the two means respectively. $n = (N_1 + N_2) - 2$, where N_1 and N_2 = number of rats from which the respective means were computed. Enter Fisher's "t" table with "n."

TABLE I

INHIBITION BY FLUORIDE OF THE DESTRUCTIVE ACTION OF CITRATE ON RATS' MOLAR TEETH

EXPERIMENT	A CITRATE-FLUORIDE I				B CITRATE-FLUORIDE II			
Drinking fluid	Citrate	Citrate + 5 ppm F	Citrate + 50 ppm F	Citrate	Citrate	Citrate + 1 ppm F	Citrate + 2.5 ppm F	Citrate + 5 ppm F
Concentration of drinking fluid	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N
pH of drinking fluid	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5
Number of rats	11	10	11	10	7	8	14	11
Group designation	Control	5 ppm	50 ppm	F Inject	Control	1 ppm	2.5 ppm	5 ppm
Days of experiment	98	98	99	98	98	97	95	92
Fluid intake per rat (ml.)	2000	1991	1992	1979	1807	1811	1815	1817
Fluorine received during test period (mg.)	0.00	9.96	99.6	49.0	0.00	1.81	4.54	9.08
Scoring of Extent of "Erosion" *Average score per rat*								
Upper right molars	5.06	2.43	3.21	4.30	4.48	4.21	3.89	2.45
Upper left molars	4.73	2.53	2.73	4.44	4.67	3.83	3.59	2.41
Lower right molars	5.15	3.17	2.61	4.60	5.19	4.21	3.76	2.55
Lower left molars	4.97	2.37	2.61	4.57	5.24	4.38	4.10	3.09
Upper molars	4.89	2.48	2.97	4.37	4.57	4.02	3.75	2.43
Lower molars	5.06	2.77	2.61	4.58	5.22	4.29	3.93	2.82
All molars	4.98	2.62	2.79	4.48	4.89	4.16	3.84	2.63

TABLE II

INHIBITION BY FLUORIDE OF THE DESTRUCTIVE EFFECT OF LACTATE ON RATS' MOLAR TEETH

EXPERIMENT	C LACTATE-FLUORIDE I				D LACTATE-FLUORIDE II			
Drinking fluid	Lactate	Lactate + 5 ppm F	Lactate + 50 ppm F	Lactate	Lactate	Lactate + 1 ppm F	Lactate + 2.5 ppm F	Lactate + 5 ppm F
Concentration of drinking fluid	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N
pH of drinking fluid	3.0-3.2	3.0-3.2	3.0-3.2	3.0-3.2	4.5	4.5	4.5	4.5
Number of rats	11	10	12	8	18	17	18	17
Group designation	Control	5 ppm	50 ppm	F Inject	Control	1 ppm	2.5 ppm	5 ppm
Days on experiment	69	74	68	70	27	29	29	29
Fluid intake per rat (ml.)	739	720	733	747	560	568	568	560
Fluorine received during test period (mg.)	0.00	3.60	36.7	35.0	0.00	0.57	1.42	2.84
Scoring of Extent of "Erosion" *Average score per rat*								
Upper right molars	4.15	2.80	2.90	5.42	2.26	2.33	1.04	1.28
Upper left molars	4.45	2.97	2.78	5.27	2.11	1.43	1.19	1.20
Lower right molars	Destruction too extensive to measure				4.83	4.80	4.65	4.47
Lower left molars					4.78	4.67	4.08	4.82
Upper molars	4.31	2.84	2.82	5.35	2.17	1.88	1.11	1.24
Lower molars	--	--	--	--	4.83	4.73	4.34	4.64
All molars	--	--	--	--	3.50	3.31	2.73	2.94

TABLE III

STATISTICAL ANALYSIS OF COMBINED DATA OF CITRATE-FLUORIDE EXPERIMENTS A AND B

GROUP DESIGNATION	AVERAGE SCORE PER RAT	NO. OF RATS
1. Control	4.95 ± 0.129*	18
2. 1 ppm	4.16 ± 0.208	8
3. 2.5 ppm	3.80 ± 0.331	14
4. 5 ppm	2.62 ± 0.213	21
5. 50 ppm	2.79 ± 0.267	11
6. Inject	4.48 ± 0.078	10
		82

Analysis of Variance
(Groups 1, 2, 3, and 4)

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	18.05	22.23	< 0.01
Regression	1	53.44	65.81	< 0.01
Deviations from regression	2	0.355	0.437	> 0.05
Within treatments	57	0.812		

*Deviations from the mean are expressed as the standard error.

TABLE IV

ANALYSIS OF THE DATA OF EXPERIMENT D

GROUP	UPPER TEETH MEAN	NUMBER OF ANIMALS	LOWER TEETH MEAN
Control	2.17 ± 0.290	18	4.83 ± 0.059
1 ppm	1.88 ± 0.282	17	4.73 ± 0.107
2.5 ppm	1.11 ± 0.180	18	4.34 ± 0.141
5 ppm	1.24 ± 0.128	17	4.64 ± 0.090
		70	

Analysis of Variance
Upper Teeth

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	44.83	5.194	< 0.01
Regression	1	9.68	10.419	< 0.01
Deviations from regression	2	2.41	2.591	> 0.05
Within treatments	66	0.93		

Lower Teeth

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	0.800	4.470	< 0.01
Regression	1	0.518	2.894	> 0.05
Deviations from regression	2	0.941	5.257	< 0.01
Within treatments	66	0.179		

RESULTS

It is apparent from Experiment A, that 50 ppm fluorine afforded no more protection against erosion by citrate at pH 6.3 to 6.5 than did 5 ppm fluorine. Another study, Experiment B, was instituted, therefore, to determine the minimum concentration of fluoride which would produce maximum protection against citrate (nonacid) erosion. It was found that increasing protection against citrate erosion occurred in the order of 1, 2.5, and 5.0 ppm fluorine. The erosion from solutions containing 5 ppm fluorine in Experiment B was essentially the same as that observed from 5 ppm fluoride solutions in Experiment A, thus indicating

that 5 ppm fluorine may be the minimum concentration affording maximum protection against erosion produced by citrate.

Analysis of the data indicates that there is a significant difference in the effect of 1 ppm, 2.5 ppm, and 5 ppm fluorine in inhibiting this type of erosion; that the inhibition is related to the dosage of fluoride as indicated by the significance of the regression line, and that the regression line adequately describes the data, i.e., the individual values adhere closely to the regression line and do not deviate from it significantly.

Application of Fisher's "t" test indicates that there is no significant difference in the erosion score between rats receiving 5 ppm and 50 ppm fluorine. It is noteworthy, however, that fluorine injected intraperitoneally appears to cause a significant reduction in erosion when compared to the Control, that is, the probability of occurrence of the observed difference between the means by chance is less than 0.01.

To determine the effect of fluoride in inhibiting acid erosion at a low pH, lactic acid at pH 3.0 to 3.2 was administered to rats. Erosion was very pronounced and destruction of the lower molars was so severe that scoring was impossible by the usual method. The upper molars, however, were scored as indicated in Experiment C, Table II. The average score per rat for Experiment C is recapitulated below:

Group	*Mean*	*No. of Rats*
Control	4.31 ± 0.398	11
5 ppm	2.84 ± 0.437	10
50 ppm	2.82 ± 0.480	12
F Inject	5.35 ± 0.232	8
		41

It will be noted that again 5 ppm fluorine afforded maximum protection, but that injected fluoride in this experiment was entirely ineffective against an acid erosion. It is difficult to explain the apparent potentiating effect of injected fluoride on erosion produced by lactate as indicated by comparing the means of the Control and F Inject groups by Fisher's "t" test. The probability of occurrence by chance of the observed difference between the means is between 0.02 and 0.05.

Inasmuch as lactic acid at pH 3.0 to 3.2 produced an erosion in the lower molars too severe to measure, Experiment D was planned to investigate the destructive effect of lactic acid at pH 4.5, and the ability of 1, 2.5, and 5 ppm fluorine to inhibit its action. The results are shown in Table II, and analysis of the data is expressed in Table IV. In view of the pronounced difference of the effect of lactate solutions at pH 4.5 on the upper and lower molars, the respective data were considered separately and were examined by the analysis of variance.

It appears that the response of the lactate-eroded molars to fluoride differs markedly from the response of citrate-eroded molars. In the latter case, both upper and lower molar teeth show the same degree of inhibition by fluoride. (See Table I.)

In the former case, however, fluoride affects the upper and lower molars differently. Analysis of the data shows that inhibition of erosion in the upper teeth by fluoride is related to its concentration as indicated by the significant slope of the regression line. Since the deviations from the regression line are not significant, a straight line adequately fits the data.

In the case of the lower teeth, however, the slope of the regression line is not significant, indicating that the degree of inhibition is not proportional to the fluoride concentration. Moreover, the deviations from the regression line are significant, indicating that a straight line does not fit the data, and that there is a "leveling-off" at 2.5 ppm fluorine.

DISCUSSION

The results of these experiments are in good agreement with previous observations[2, 3, 4] in showing, particularly, that the rat erosion-inhibitory property of fluorine is operative within a relatively narrow range and at a very low concentration of fluoride (1 to 5 ppm fluorine). In Experiment D, Table II, 2.5 ppm fluorine was as effective as 5 ppm in the lower teeth, and in Experiments A and C, 50 ppm fluorine was no more effective than 5 ppm fluorine. This latter result applied to both lactate (acid) and citrate (nonacid) solutions.

It is known from previous studies in this laboratory that both injected fluoride[17] and orally ingested fluoride[18] add fluorine posteruptively to rats' molar dentin and enamel. The amount incorporated is proportional to the quantity of fluorine being ingested in the drinking water. Samples of molar dentin and enamel of the rats of Experiment A were analyzed for fluorine in this study and the results confirm this posteruptive addition of fluorine. Owing to the very small quantities of enamel available for analysis, the results were accepted with reservation. They indicate, however, that there was approximately 5 times more fluorine in both the molar dentin and enamel of the rats receiving 50 ppm fluorine than in the rats receiving 5 ppm fluorine, as shown in Table V.

TABLE V

THE EFFECT OF ADMINISTRATION OF FLUORIDE ON THE FLUORINE CONTENT OF INCISOR AND MOLAR DENTIN AND ENAMEL

EXPERIMENT A

GROUP	MG. F CONSUMED	INCISORS		MOLARS	
		DENTIN	ENAMEL	DENTIN	ENAMEL
Control	0.00	0.0026	0.0022	0.0052	0.0015
5 ppm	9.96	0.0261	0.0107	0.0269	0.0075
F Inject	49.1	0.0703	0.0193	0.0854	0.0078
50 ppm	99.6	0.2000	0.0694	0.1665	0.0407

When fluoride was injected intraperitoneally, increased amounts were found in the incisor and molar dentin and enamel. It should be noted, however, that the increase of fluorine in the enamel was much less than in the dentin. This was particularly true in the case of molar enamel where only a slight increase in the fluorine content was observed as compared to the 5 ppm group, although the former received five times more fluoride. This finding has previously been reported by Arnold and McClure.[17]

Although more fluorine was incorporated in the enamel of the 50 ppm group than in the 5 ppm group of Experiment A, the degree of rat erosion was essentially the same in both groups. It seems probable, therefore, that the quantity of fluorine in the dentin and enamel above that contributed by 5 ppm fluorine in the drinking water has no further preventive influence on rat erosion.

It is of interest to note that the inhibitory effect of fluorine on enamel erosion in rats is operative at much lower concentrations than is the inhibitory effect of fluorine on induced rat caries. In the latter case, fluoride became partially inhibitory at 10 ppm fluorine, was increasingly effective at 50 ppm, and was only fully caries-inhibitory at 100 and 125 ppm fluorine.[18] With respect to the quantitative relations, our rat-erosion results suggest some similarity to the inhibitory effect of fluoride on human dental caries. Thus, Dean[19] has pointed out that a marked reduction in human dental caries occurs at 1.0 to 1.5 ppm fluorine in the drinking water, but further increases in water-fluorine concentration seemingly afford little further caries reduction.

More pertinent to our erosion studies, and illustrative also of the absence of a strictly quantitative relation between fluorine and its effect on enamel, are recent observations by Muhler and Van Huysen.[20] Within the limits of their studies based on measurements of weight loss of powdered enamel, it was observed that a 4.0 per cent sodium fluoride solution gave essentially no more protection against enamel decalcification in vitro than did a 0.2 per cent solution.

It seems highly probable that the inhibitory effect of fluoride on acid erosion in rats is due to a decrease in enamel solubility, but it is noteworthy also that the antisolubility effect of fluorine in vivo seems to have a limit of effectiveness, which is about 5 ppm of orally ingested fluorine. Further study is needed to explain the effect of fluorine on nonacid citrate erosion.

It is difficult to explain the anomalous effect of lactate on rats' molar teeth. This observation has previously been made by Gortner, Restarski, Bieri, and McCay[3] and by McClure and Ruzicka.[6]

In conclusion, it should be noted that injected fluoride also appeared to have an inhibitory effect on erosion caused by citrate as shown by analysis of the data of Table III using Fisher's "t" test. The inhibitory effect of the injected fluoride (49.1 mg.), however, is produced also by only 1 ppm of orally administered fluoride (1.81 mg.).

Injected fluoride had no effect in reducing the erosion produced by lactate solutions as shown in Experiment C, Table II. Further studies are contemplated to study the effect of injected fluoride in inhibiting acid (lactate) as well as nonacid (citrate) erosion.

SUMMARY

1. In rats' molar teeth, 2.5 ppm fluorine gave maximum protection against erosion by lactate at pH 4.5, and 5 ppm fluorine afforded maximum protection against erosion by citrate at pH 6.3 to 6.5. As little as 1 ppm fluorine is effective in significantly reducing the erosion produced by lactate at pH 4.5 and by citrate at 6.3 to 6.5. A minimum concentration of fluoride exists, beyond which further increases in administered fluorine afford no further protection against dental erosion in rats.

2. Fluorine acquired by intraperitoneal injection appeared to be effective in reducing the erosion produced by citrate. However, more than twenty-five times more fluorine was necessary to give the same effect produced by 1 ppm fluorine orally administered.

3. Fluorine injected intraperitoneally was ineffective in reducing the amount of erosion produced by lactate.

We are indebted to Mr. Jerome Cornfield, Statistician, Division of Public Health Methods, Office of The Surgeon General, USPHS, for statistical treatment of the data.

REFERENCES

1. McClure, F. J.: The Destructive Action in Vivo, of Dilute Acids and Acid Drinks and Beverages on the Rats' Molar Teeth, *J. Nutrition* **26**: 251, 1943.
2. Restarski, J. S., Gortner, R. A., Jr., and McCay, C. M.: Effect of Acid Beverages Containing Fluorides Upon the Teeth of Rats and Puppies, *J. A. D. A.* **32**: 668, 1945.
3. Gortner, R. A., Jr., Restarski, J. S., Bieri, J. G., and McCay, C. M.: Factors Influencing the Destructive Effects of Acidic Beverages on the Teeth of White Rats and Hamsters, *Arch. Biochem.* **8**: 405, 1945.
4. Bieri, J. G., McCay, C. M., Restarski, J. S., and Gortner, R. A., Jr.: Further Studies on in Vivo Decalcification by Acid Beverages, *Arch. Biochem.* **11**: 33, 1946.
5. Wynn, W., and Haldi, J.: The Erosive Action of Various Fruit Juices on the Lower Molar Teeth of the Albino Rat, *J. Nutrition* **35**: 489, 1948.
6. McClure, F. J., and Ruzicka, S. J.: The Destructive Effect of Citrate vs. Lactate Ions on Rats' Molar Tooth Surfaces, in Vivo, *J. D. Res.* **25**: 1, 1946.
7. Gortner, R. A., Jr., McCay, C. M., Restarski, J. S., and Schlack, C. A.: Some Effects of Dietary Oxalate on the Teeth of White Rats, *J. Nutrition* **32**: 121, 1946.
8. Hartmann, B. G., and Hillig, F.: Acid Constituents of Food Products, *J. Assoc. Off. Agri. Chem.* **17**: 522, 1934.
9. Greenwald, I.: The Dissociation of Some Calcium Salts, *J. Biol. Chem.* **124**: 457, 1938.
10. Shear, M. J., and Kramer, B.: Composition of Bone. V. Some Properties of Calcium Citrate, *J. Biol. Chem.* **79**: 161, 1928.
11. Shear, M. J., Kramer, B., and Resnikoff, L.: Composition of Bone. VIII. Conductivity Titrations of Calcium Ion With Chloride, Acetate, Lactate and Citrate Ions at 38°, *J. Biol. Chem.* **83**: 721, 1929.
12. Hastings, A. B., McLean, F. C., Eichelberger, L., Hall, J. L., and Da Costa, E.: The Ionization of Calcium, Magnesium and Strontium Citrates, *J. Biol. Chem.* **107**: 351, 1934.
13. Stephan, R. M.: Intra-oral Hydrogen-ion Concentrations Associated With Dental Caries Activity, *J. D. Res.* **23**: 257, 1944.
14. Muntz, J. A.: Production of Acids From Glucose by Dental Plaque Material, *J. Biol. Chem.* **148**: 225, 1943.
15. Restarski, J. S., Gortner, R. A., Jr., and McCay, C. M.: A Method of Measuring the Effects of Acid Beverages on the Teeth of Small Laboratory Animals, *Science* **102**: 404, 1945.
16. Fisher, R. A.: Statistical Methods for Research Workers, ed. 8, London, 1941, Olivier & Boyd.
17. Arnold, F. A., Jr., and McClure, F. J.: Observations on Induced Dental Caries in Rats. II. The Effect of Subcutaneous Injection of Fluoride, *J. D. Res.* **20**: 457, 1941.
18. McClure, F. J.: Observations on Induced Caries in Rats. III. Effect of Fluoride on Rat Caries and on Composition of Rats' Teeth, *J. Nutrition* **22**: 391, 1941.
19. Dean, H. T.: Epidemiological Studies in the United States, in Dental Caries and Fluorine, Publication of the Am. Assoc. Adv. Sci., Lancaster, Pa., 1946, Science Press.
20. Muhler, J. C., and Van Huysen, G.: Sodium Fluoride Reagent Concentration and Powdered Enamel Solubility, *J. D. Res.* **27**: 142, 1948.

SUBSTANCES CAPABLE OF DECREASING THE ACID SOLUBILITY OF TOOTH ENAMEL

R. S. MANLY* AND B. G. BIBBY†

From the Chemical Division, the Procter and Gamble Company, Ivorydale, Ohio, and Tufts College Dental School, Boston, Mass.

IN 1939 Volker presented a method for testing the rate of solubility of tooth enamel in acid, and showed that powdered tooth enamel became less soluble after a few minutes' treatment with various concentrations of sodium fluoride solution.[1] This finding was considered by him to be important because it might explain the mechanism by which fluoride in certain water supplies is able to decrease the incidence of dental caries.

The influence of several other salts on the acid solubility of tooth enamel has been studied. W. D. Miller[2] was unable to observe any protective influence of silver nitrate solution when whole teeth were employed and the effect was observed on ground sections. Hill and Arnold[3] found that the solubility of powdered enamel was decreased by treatment with silver nitrate solutions. Buonocore and Bibby[4, 5] tested the effect of nine salts in reducing the acid solubility of tooth enamel powder. Silver nitrate decreased the acid solubility of powdered enamel only slightly. Cooper, zinc, and lead ions were found to be effective and lead fluoride was especially active. Lead nitrate and lead fluoride were more effective than sodium fluoride. Muhler and Van Huysen[6] reported on the influence of thirteen substances, eight of which had not been previously studied. The effectiveness of sodium fluoride, lead fluoride, lead nitrate, silver nitrate, and copper sulfate were confirmed. Stannous fluoride was found to be more effective than the lead fluoride, and the remaining seven substances decreased acid solubility to some extent. Rae and Clegg[7] confirmed the solubility-depressing effect of sodium fluoride, ammonium oxalate, lead nitrate, and silver nitrate, and added barium nitrate and silver fluoride to the list of those substances which had been reported to be active.

In the present work 147 substances, representing compounds of fifty-seven elements, were tested by one or both authors working independently in different laboratories. Investigations were begun early in 1944 and were conducted independently by each of the authors without knowledge of the other's interest in the subject. The aim in each case was to make a search for substances other than fluorides which might also have similar solubility-depressing effects on tooth enamel solubility. There were minor differences in technic of acid solubility measurement. One author (RSM) usually employed the salt being tested at a concentration of 1 per cent in water; the other (BGB) generally used the salt at $\frac{1}{10}$ per cent concentration dissolved in an acetic acid buffer. After the publication by Bibby and Buonocore in December, 1944, and in April, 1945, each author was made aware of the other's interest in this work but the studies nevertheless continued independently. Because there was duplication in the majority of substances tested, the authors decided to present the findings jointly.

Received for publication September 28, 1948.

*Present address: Tufts College Dental School, 416 Huntington Ave. Boston, Mass.
†Present address: Eastman Dental Dispensary, Rochester, N. Y.

PROCEDURE

A. *Technics Employed by R. S. M.*

The preparation of powdered enamel by one of us (RSM) was carried out by a modification of a method that has been reported.[8] Several hundred extracted teeth were scraped free of deposits, dried in air, and cracked into fragments in a diamond mortar. The fragments were pulverized in a motor-driven food mill until the powder would pass an 80-mesh screen. The portion between 80 and 200 mesh was washed with carbon tetrachloride, dried in air, and immersed in bromoform in a graduated cylinder. The powder was stirred, the enamel permitted to settle out, and the dentin at the top of the liquid was scooped off with a piece of wire screen. The enamel was washed with acetone, dried in air, and the iron particles removed with a magnet. At this stage of preparation, the purity of enamel was found to be 85 per cent according to the Becke line test.[8] In order to improve the purity, the enamel was again immersed in bromoform and dentin floating on top of the liquid was removed. The purity at this stage was 97 per cent and was considered to be satisfactory.

The acid solubility of purified enamel was determined by a slight modification of the method of Volker.[1] Exactly 50 mg. of enamel were placed into a large test tube, 10 ml. of an acetate buffer having a pH of 4 were added and the powder stirred with a mechanical stirrer for 15 minutes. Gooch crucibles were used for collecting the treated enamel because of the unavailability of the brand recommended by Volker. The tubes and stock bottles of buffer solution were kept immersed in a water bath at 40° Centigrade. The enamel was decanted and washed into a tared crucible, tried for two hours in an oven at 100° C., cooled, and reweighed.

Solutions to be tested were stirred with the enamel powder for three minutes, centrifuged, and the liquid decanted. The acetate buffer was added and the test for acid solubility was carried off as described. Duplicate determinations were run in every case and the average of the results calculated. In case the amounts dissolved differed by more than one mg., a second set of duplicates was run, and the average reported separately.

For part of the tests, Selas crucibles were substituted for the Gooch crucibles. After a few months, however, the results with Selas crucibles began to deviate widely and the difficulty was traced to entrapment of powder in grooves which developed because of solution of cement holding the plate at the bottom of the crucible. Hence, Gooch crucibles were once again employed. Some of the tests were made with a sixty-minute period for acid treatment, but it was soon discovered that a fifteen-minute period was sufficient.

Substances for test were selected from a list of sodium and potassium salts which were listed in a chemical handbook. All were investigated in water solution at concentrations of 1.0 or 0.1 per cent unless otherwise noted. The lower concentration was generally employed for those substances which were only sparingly soluble or known to have an undesirable taste or fair toxicity. An attempt was made to obtain representative compounds of all elements which are available commercially.

B. *Technics Employed by B. G. B.*

In broad outline, the experimental procedures used were the same as those used by the other investigator (RSM), but differed in the following ways: After the teeth were cleaned with an engine brush, the carious areas were removed, the tooth root was cut off, and the pulp chamber contents were extirpated. The crowns of the teeth were then powdered fine enough to pass a 100-mesh screen. In the solubility tests 100 mg. samples of enamel were used in most instances, although occasionally 50 mg. samples were employed. The whole test procedure was carried out in Berliner filter crucibles set into a suction chamber. After the samples had been weighed in the crucibles, 10 or 20 ml. of the reagent were added, and stirred mechanically for 20 minutes. The reagent was sucked through the filter and the enamel washed with distilled water. The decalcifying buffer was added, stirred, and drawn off. The crucible was washed and dried for two hours at 105° Centigrade. All tests were carried out in duplicate and discordant results discarded. In some series of tests, an additional drying and weighing was made after the reagent was applied. Care was taken to check the efficiency of the Berliner crucibles frequently. Those not passing water at a satisfactory rate or not maintaining constant weight were discarded. Reagents were chosen so that the coverage of metals in the periodic table would be as broad as possible. Salts were obtained from the chemistry department of Tufts College or from the suppliers of rare chemicals.

RESULTS

A total of 147 substances have been tested for their effect on the acid solubility of enamel. These substances have been compiled alphabetically in Table I, in order of the ions being tested. The other ions present, the solvent, and the concentration of the salt are recorded in the second, third, and fourth columns of the table. The solubility reduction findings are given as percentage figures with negative values indicating increased solubility. The control solubility equals zero, complete insolubility equals 100, and values greater than 100 indicate an actual increase in weight of enamel powder. Here, as elsewhere, data obtained by each author are presented separately. Most of the substances were inactive and Table I serves chiefly for purpose of record.

Those substances which reduced the acid solubility by 10 per cent or more are included in Table II in order of their activity and are separated into groups according to whether or not they are more active than 0.1 per cent sodium fluoride. This substance was chosen as the dividing line because it had been tried experimentally in topical application. Those substances which are less active than 0.1 per cent sodium fluoride might be of practical interest if they possessed other advantages such as lesser toxicity or greater tendency to produce a durable protective layer. When there were several values for one substance, depending on concentration or solvent, the one indicating greatest inhibition was selected. In case of variation in results at the same concentration and solvent, an average was taken.

A total of seventy-four substances were found which reduced the acid solubility of enamel by 10 per cent or more. Twenty-seven of these were more active than sodium fluoride. The most active substance was lead fluoborate which undoubtedly deposited a coating on the enamel for the weight after the acid treatment was greater than the original weight. It is evident that the activity of fluoride is considerably influenced by the other ions present. Lead fluoborate, indium fluoborate, lead fluoride, hydrogen fluoride, ammonium bifluoride, potassium titanium fluoride, and strontium, zinc, and barium fluorides were all more active than sodium fluoride. On the other hand, fluoride salts of silver, iron, boron, calcium, and aluminum, were less active than sodium fluoride.

Possibly the coordinated FHF ion is needed for maximum activity of fluoride. This would be present in hydrogen fluoride, ammonium bifluoride, and other acid salts of fluoride. Fluorides in acid have the property of etching glass and this led us to compare the properties of 0.1 per cent NH_4HF_2 solution when stored in soft glass and paraffin-coated bottles. The solution in glass developed a precipitate, the pH rose from 3.5 to 4.5 in ten days and at the end of this period, the solution reduced the acid solubility of enamel by 53 per cent. On the other hand, the portion which was stored in paraffin over the same period remained clear, did not change in pH, and was able to reduce the acid solubility of enamel 70 per cent. If acidified fluoride solutions are to be used in topical applications,* they should not be stored in soft glass bottles.

From this table certain cations appear to be promising as agents for reducing enamel solubility. These include gallium, yttrium, indium, iron, and thorium salts. The practical possibilities will depend upon toxicity, cost, and relative permanence of the effect. The resistance imparted by certain of the ions, notably beryllium, pyrophosphate, and tripolyphosphate, are readily rinsed off as will be mentioned in a later publication by one of us.

DISCUSSION

A. *Agreement Between Results of Different Investigators*

It is surprising that there are only three instances in Table I in which the same substance was tested in the same solvent at the same dilution by both authors. With sodium fluoride and potassium titanium fluoride in aqueous solution there was agreement, but with aluminum chloride the findings differed by nearly 13 per cent. A similar situation exists when data obtained by the authors are compared with that previously reported by others. The findings by others were converted into the percentage of control solubility in order to be comparable with the present work. Our results are in excellent agreement with values reported for NaF,[1] PbF_2,[6] and ZnF_2.[6] There is fair agreement with tests on $CuSO_4$[6] and a second investigator's value for NaF.[6] There is poor agreement with data reported for AgF,[7] NaI,[6] $NaIO_3$,[6] $AgNO_3$,[3, 6, 7] and a third investigator's value for NaF.[9] The findings on $AgNO_3$ are quite divergent. It is probable that agreement between investigators cannot be expected because the tech-

*It is the authors' opinion that the use of acidulated solutions is contraindicated (BGB, *J. D. Res.* 27: 497, 1948.)

nics are dissimilar. Several of the differing factors may have considerable effect upon the observed solubility of enamel. These include the treatment of the enamel in preparation of enamel powder, the particle size of the powder, and the quantities of trace elements which are already present in the enamel. There are several influences which are related to the testing procedure. A greater reduction in acid solubility would usually be expected when the concentration of reagent is high during the test, when the time of treatment is long, and when the acid-solubility test period is short. These generalizations should hold for neutral test solutions. If the solutions were fairly acid, higher concentration and longer treatment time might actually decrease the effectiveness. If work by one author is compared with that of the other, there are several other substances which appear to act inversely. These include magnesium chloride, strontium chloride, rhodium chloride, rubidium chloride, and ruthenium chloride.

Concentrated solutions of lead fluoride, lead fluoborate, yttrium nitrate, and sulfate reduced enamel solubility more than dilute solutions of the reagents. The effect of silver nitrate was independent of concentration within the range studied. On the other hand, concentrated solutions of indium fluoborate, zinc fluoborate, rubidium chloride, and potassium perrhenate were less effective than dilute solutions.

B. *Analysis According to the Periodic Table*

The distribution of active ions among different groups or series of the periodic table has been considered. Elements of Groups III and IV of the periodic table seem to be most important in their ability to depress the acid solubility of enamel. Of the nine members of Group III which have been tested in cationic form, all have been active. These include five of the rare earths—lanthanum, cerium, samarium, dysprosium, and "didymium"—and aluminum, gallium, indium, and yttrium. Only borate was inactive. Yttrium is one of the more abundant metals which are classed as rare earths. Gallium and indium are less common. Nine rare earths, plus scandium and actinium, have not been tried because samples were unavailable.

Among Group IV elements, titanium, zirconium, tin, lead, and thorium are active in cationic form, and germanate in anionic form. Silicon is slightly active in the form of metasilicate. Carbonate, orthosilicate, titanate, and stannate were inactive.

In Group I, lithium, copper, rubidium, and gold are active but not sodium and potassium. There is some question concerning the activity of caesium and silver. Among Group II elements beryllium is very active, and mercury, zinc, barium, strontium, calcium, and cadmium are slightly active; only magnesium was inactive.

Vanadium, arsenic, columbate, certain polyphosphates, and a polyvanadate were active among Group V elements, but nitrate, phosphate, orthovanadate, arsenate, and arsenite were not. Antimony, tantalum, bismuth, and proactinium were not tested. Hydrosulfite, chromium, molybdate, and uranium were active from Group VI compounds which were tested, and sulfate, sulfite, thiosulfate, chromate, dichromate, and selenate were not; tellurium, tungsten and polonium were not tried.

Fluoride is the only active element among those of Group VII and is the only single element anion which has an effect. Ten representatives of Group VII were tried.

Among the Group VIII or transitional elements, iron is active but only in trivalent form. Ferric ammonium sulfate is very effective while ferrous ammonium sulfate is completely inactive. Cobalt, ruthenium, and iridium also have some effect.

Elements in cationic form are more likely to be active than those in anionic form. Aluminum, chromium, iron, gallium, tin, titanium, and vanadium are much less active as anions than as cations. This is in accord with the theory that many of the active substances replace calcium from combination with phosphate of tooth enamel.

C. *Presence of Active Elements in Minerals Containing Phosphate*

Phosphate is found in nature in combination with many different elements. It seems probable that those minerals which contain phosphate would be made up of the least soluble compounds which could be formed from the ions that were present in solution at the time the mineral was formed. The least soluble compounds will be the first to precipitate from solution, and when minerals are subject to leaching by water, the least soluble will be the last to dissolve. Both of these tendencies would have the effect of causing phosphate to be found in combination in nature with those ions which impart a low solubility to the compound. It seemed worth while to examine whether or not the elements found associated with phosphate in nature were the same as those which are capable of reducing the acid solubility of tooth enamel. If this were the case, it would suggest that the mechanism for reducing the acid solubility of enamel consisted of combination between phosphate and the effective cations.

A list of minerals which included qualitative analysis was examined.[10] It was found that there were forty-five minerals containing phosphate. All but three of these contained one or more elements which are capable of decreasing acid solubility of tooth enamel. Twenty-four of these contained two or more such ions. Altogether, there were more than sixteen active elements represented (Al, Be, Ba, Ce, Cu, Di, Fe, F, La, Li, Pb, Y, U, and rare earths). Seven of these were more active than 0.1 per cent NaF.

Analyses of teeth and saliva indicate that traces of active elements are found in both. Drea[11] identified Ag, Al, Ba, Cu, Fe, Pb, Si, Sr, Ti, U, and Zn in teeth. All of these except Si are able to depress acid solubility. Lowater and Murray[12] found that B, Cr, Mn, Na, Ni, and Sn were also present in addition to the elements mentioned above. Of this new list of elements only tin has any appreciable activity. From analysis by Brown and Kitchin[13] the presence of Ag, Al, Cu, Fe (trace), Pb, Si, Na, and K were found in saliva. Five of these elements are active and their continued presence might cause the gradual decrease in acid solubility of teeth which has been observed after eruption.[14]

The adult enamel used in our solubility tests was further reduced in acid solubility by the effective salts, probably because the enamel had absorbed only a small fraction of the amounts necessary to produce saturation.

TABLE I

SUBSTANCE OR ION	OTHER ION PRESENT	% OF COMPOUND IN SOLUTION	SOLVENT	SOLUBILITY REDUCTION (CONTROL SOLUBILITY = 0)	
				RSM	BGB
Aluminate	Na	0.1	H_2O	3	–
Aluminum	Cl_3	0.1	H_2O	18	31
Aluminum	$(SO_4)_3$	1.0	H_2O	22	–
Aluminum	$K(SO_4)_2$	0.1	H_2O	16	–
Ammonium	Cl	1.0	H_2O	–10	–
	SO_4	1.0	H_2O	– 4	–
Arsenate	Na_3	0.1	H_2O	– 1	–
Arsenite	Na_2H	0.1	H_2O	1	–
Arsenic	Oxide (AsO_3)	0.005	H_2O	–	49
Barium	Cl_2	1.0	H_2O	14	–
Barium	Cl_2	0.1	H_2O	–	16
Barium	F_2	0.1	Ac	–	51
Benzoate	Na	1.0	H_2O	1	–
Beryllium	Cl_2	Sat'd (0-1%)	H_2O	69	–
	NO_3	1.0	H_2O	60, 54	–
Boron	F_3	0.1	H_2O	21, 16	–
Borate	Na_2	Sat'd (<1.0)	H_2O	1	–
Bromate	Na	0.1	H_2O	5	–
Bromide	Na	0.1	H_2O	– 1	–
Cadmium	$(NO_3)_2$	1.0	H_2O	11	–
Caesium	NO_3	1.0	H_2O	– 7	–
Caesium	Cl	0.1	H_2O	–	35, –6
Calcium	Cl_2	1.0	H_2O	4	–
Calcium	Cl_2	0.1	H_2O	–	11
Carbonate	Na_2	1.0	H_2O	– 1	–
Cerium	$(NO_3)_3$	Sat'd (<1.0)	H_2O	69	–
Chlorate	K	1.0	H_2O	3	–
Chloride	Na	0.1	H_2O	– 6	–
Chloride	Na	0.1	Ac	–	[illegible]
Chloride	K	0.1	H_2O	–	4
Chlorite (Hypo)	Na	0.1	H_2O	– 6	–
Chromate	Na_2	0.1	H_2O	0	–
Chromium	Ac	0.1	Ac	–	10
Citrate	Na_3	1.0	H_2O	2	–
Cobalt	$(NO_3)_2$	1.0	H_2O	– 2, –8	–
Cobalt	$(NO_3)_2$	0.1	Ac	–	24
Columbiate	K	1.0	H_2O	4	–
Columbiate	K	0.1	H_2O	–	20
Copper	SO_4	1.0	H_2O	28, 24	–
Copper	SO_4	0.1	Ac	–	34, 26
Dichromate	Na_2	0.1	H_2O	5	–
"Didymium"	Cl_3	1.0	H_2O	19	–
Dysprosium	$(NO_3)_3$	0.005	H_2O	–	56
Ferric	$NH_4(SO_4)_2$	1.0	Ac	55	–
Ferric	$(SO_4)_3$	0.1	Ac	–	18
Ferric	Cl_3	1.0	H_2O	52, 14	–
Ferric	Cl_3	0.1	H_2O	–	35
Ferrous	$(NH_4)_2(SO_4)_2$	1.0	H_2O	6	–
Ferricyanide	K_3	1.0	H_2O	– 5	–
Ferrocyanide	K_4	1.0	H_2O	–22	–
Fluoborate	Na	0.1	H_2O	32	–
Fluoborate	Na	0.03	H_2O	27, 24	–
Fluoride	Ag	0.1	Ac	–	36
Fluoride	Al	<0.1	H_2O	15	–
Fluoride	Ba	0.1	Ac	–	51
Fluoride	Ba	<0.1	H_2O	39	–
Fluoride	B	0.1	H_2O	21, 16	–
Fluoride	Ca	<0.1	H_2O	20, 17	–
Fluoride	Ca	0.1	Ac	–	14
Fluoride	Fe	0.1	Ac	–	33
Fluoride	H	0.1	H_2O	71	–

TABLE I—CONT'D

SUBSTANCE OR ION	OTHER ION PRESENT	% OF COMPOUND IN SOLUTION	SOLVENT	SOLUBILITY REDUCTION (CONTROL SOLUBILITY = 0)	
				RSM	BGB
Fluoride	Na	0.1	H_2O	42, 37	50, 41
Fluoride	Li	0.1	Ac	–	19
Fluoride	NH_4H	0.1	H_2O	70	–
Fluoride	Pb	0.1	Ac	–	86, 85, 84, 79
Fluoride	Pb	0.06	H_2O	–	60
Fluoride	Pb	0.04	Ac	–	62
Fluoride	Pb	0.02	Ac	–	52
Fluoride	Sr	0.1	Ac	–	54
Fluoride	K_2Ti	0.1	H_2O	57	51
Fluoride	Zn	<0.1	H_2O	47	–
Fluoride	Zn	0.1	Ac	–	54
Fluorobenzene	–	0.1	1% "Dreft"	2	–
Gallium	$(NO_3)_3$	1.0	H_2O	85	–
Gallium	Cl_3	0.1	H_2O	–	88
Galliate	K	1.0	Dil. KOH	36	–
Germanate	K	0.005	Dil. KOH	–	41
Gold	Cl_3	0.1	Ac	–	29
Guanidine	Ac	1.0	H_2O	– 3	–
Indium	Cl_3	1.0	H_2O	47	–
Indium	Cl_3	0.1	H_2O	–	73
Indium	$(NO_3)_3$	0.005	H_2O	–	81
Indium	$(BF_4)_3$	2.5	Ac	–	–52
Indium	$(BF_4)_3$	2.5	H_2O	–	–120
Indium	$(BF_4)_3$	0.5	H_2O	–	34
Indium	$(BF_4)_3$	0.05	H_2O	–	84
Iodate	Na	0.1	H_2O	– 2	–
Iodide	Na	0.1	H_2O	– 2	–
Iridium	Cl_4	0.1	H_2O	–	48
Lanthanum	Cl_3	1.0	H_2O	23	–
Lanthanum	Cl_3	0.1	H_2O	–	22
Lead	Ac	1.0	H_2O	75	
Lead	F	(See Fluoride)	H_2O		
Lead	$(BF_4)_2$	–	Ac	–	140
Lead	$(NO_3)_2$	0.1	Ac		68, 72
Lead	$(BF_4)_2$	2.5	H_2O	–	165
Lead	$(BF_4)_2$	0.5	H_2O	–	175
Lead	$(BF_4)_2$	0.05	H_2O	–	109
Lead	$(BF_4)_2$	0.005	H_2O	–	104
Lead	$(BF_4)_2$	5.0	H_2O_2	–	91
Lead	$(BF_4)_2$	2.0	H_2O_2	–	117
Lead	$(BF_4)_2$	0.5	H_2O_2	–	113
Lithium	Cl	0.1	H_2O	–	37, 32
	F	0.1	Ac	–	19
Lithium	Cl	1.0	H_2O	– 2	–
Magnesium	Cl_2	1.0	H_2O	7	–
Magnesium	Cl_2	0.1	H_2O	–	– 1
Manganese	Cl_2	1.0	H_2O	5	–
Manganese	SO_4	0.1	Ac	–	–40
Mercuric	Cl_2	1.0	H_2O	16, –3	
Mercuric	Cl_2	0.1	H_2O	–	36
Mercuric	Cl_2	0.1	Ac	–	2
Metaborate	Na	1.0	H_2O	5	–
Methenamine	–	4.0	H_2O	– 2	–
Nickel	Cl_2	1.0	H_2O	1	–
	(NO_3)	0.1	Ac	–	+ 4
Nitrate	Na	1.0	H_2O	– 4	
Nitrite	Na	0.1	H_2O	2	–
Nitroprusside	Na_2	1.0	H_2O	– 3	–
"Nullapon B"	–	1.0	H_2O	–13	–
Oleic acid	–	1.0	3% "Dreft"	19	–
Oxalate	Na_2	0.1	H_2O	3	–

TABLE I—CONT'D

SUBSTANCE OR ION	OTHER ION PRESENT	% OF COMPOUND IN SOLUTION	SOLVENT	SOLUBILITY REDUCTION (CONTROL SOLUBILITY = 0)	
				RSM	BGB
Oxalate	Na_2	0.1	Ac	–	42, 39, 38
Perborate	Na	1.0	H_2O	- 1	–
Permanganate	K	0.1	H_2O	- 4	–
Perrhenate	K	0.1	H_2O	–	28
Perrhenate	K	1.0	H_2O	- 6	–
Phosphates					
Glycerophosphate	Na_2	0.1	H_2O	0	–
Orthophosphate	NaH	1.0	H_2O	- 2	–
Orthophosphate	Na_2H	1.0	H_2O	2	–
Orthophosphate	Na_3	1.0	H_2O	0	–
Pyrophosphate	Na_4	1.0	H_2O	43, 54, 47	–
Pyrophosphate	Na_2H_2	1.0	H_2O	55	–
Pyrophosphate	K_4	1.0	H_2O	39	–
Metaphosphate	Na	<1.0 (Sat'd)	H_2O	12	–
Trimetaphosphate	Na	1.3	H_2O	22	–
Tripolyphosphate	Na	1.0	H_2O	56	–
Hexametaphosphate	Na	1.0	H_2O	60, 36	–
Phosphatide	-	0.1	H_2O	- 9	–
Phosphatidate	Na	Sat'd	H_2O	- 1	–
Phosphite, Hypo	Na	0.1	H_2O	- 4	–
Phytate	Ca	Sat'd (<1.0)	H_2O	23	–
Potassium	Cl	0.1	H_2O	–	4
Rhodium	Cl_3	1.0	H_2O	-44	–
Rhodium	Cl_3	0.1	H_2O	–	27
Rhodizonate	Na	0.1	H_2O	6	–
Rubidium	Cl_2	1.0	H_2O	- 5	–
Rubidium	Cl_2	0.1	H_2O	–	20
Rubidium	Cl_2	0.005	H_2O	–	47
Ruthenium	Cl	1.0	H_2O	-147	–
Ruthenium	Cl	0.1	H_2O	–	29
Salicylate	Na	0.1	H_2O	- 5	–
Samarium	Cl_3	1.0	H_2O	–	–
Samarium	Cl_3	0.1	H_2O	15	–
Selenate	Na_2	0.1	H_2O	- 5	28
Silver	NO_3	1.0	H_2O	17, –1	–
Silver	NO_3	0.1	H_2O		7
Silver	F	0.1	H_2O	–	36
Silicate	Na_2	1.0	H_2O	3, –6	–
Silicate, Meta	Na_2	1.0	H_2O	11, –8	–
Silocofluoride	Na_2	0.1	H_2O	46, 25, 24	–
Sodium	Cl	1.0	H_2O	- 6	–
Sodium	Cl	0.1	Ac	–	6
Sodium ?	SO_4	1.0	H_2O	- 2	–
Sodium	SO_4	0.1	Ac	–	0
Stannate	Na_2	1.0	H_2O	3, 2	–
Strontium	Cl_2	1.0	H_2O	8	–
Strontium	Cl_2	0.1	H_2O	–	13
Strontium	Cl_2	0.1	Ac	–	15
Strontium	F_2	0.1	Ac	–	55
Sulfate	Na_2	1.0	H_2O	- 2	–
Sulfate	Na_2	0.1	Ac	–	0
Sulfite	Na_2	1.0	H_2O	1	–
Sulfite, Hydro	Na_2	0.1	H_2O	24	–
Tartrate	NaK	1.0	H_2O	8	–
Thiocyanate	K	1.0	H_2O	0	–
Thiosulfate	Na_2	1.0	H_2O	1	–
Tin	Cl_2	0.1	H_2O	–	43
Thorium	$(NO_3)_4$	1.0	H_2O	52	–
Thorium	$(NO_3)_4$	0.005	H_2O	–	48
Titanate	K_2	Sat'd (<0.1)	H_2O	6	–
Titanium	$(NO_3)_4$	0.005	H_2O		43

TABLE I—CONT'D

SUBSTANCE OR ION	OTHER ION PRESENT	% OF COMPOUND IN SOLUTION	SOLVENT	SOLUBILITY REDUCTION (CONTROL SOLUBILITY = 0)	
				RSM	BGB
Titanium	$(NO_3)_4$	<0.1	H_2O	- 9	-
Titanofluoride	K_2	0.1	H_2O	57	51
Thallium	Ac	0.1	H_2O	-	31
Thallium			Ac	-	3
Uranium	Ac	<1.0	H_2O	7	-
Uranium		0.1	H_2O	-	24
Uranium		0.1	Ac	-	22
Urea	-	40.0	H_2O	- 4	-
Urea	-	4.0	H_2O	2	
Vanadate, Ortho	Na	1.0	H_2O	7	-
Vanadate, Meta	Na	1.0	H_2O	18	-
Vanadium	Cl_3	(<0.1)	H_2O	46	
Vanadium	O_5	0.005	H_2O	-	41
Yttrium	$(NO_3)_3$	Sat'd (<1.0)	H_2O	89, 81	
Yttrium	$(NO_3)_3$	½ Sat'd	H_2O	35	
Yttrium	$(NO_3)_3$	¼ Sat'd	H_2O	11	-
Yttrium	$(SO_4)_3$	.05	H_2O	-	57
Yttrium	$(SO_4)_3$	0.10	H_2O	-	68, 58
Zephiran	-	0.1	H_2O	0	-
Zinc	Cl_2	0.1	H_2O	18	-
Zinc	$(NO_3)_2$	0.1	Ac		18
Zinc	F_2	0.1	Ac	-	54
Zinc	$(BF_4)_2$	0.05	H_2O	-	15
Zinc	$(BF_4)_2$	0.5	H_2O	-	- 5
Zinc	$(BF_4)_2$	2.5	H_2O	-	-30
Zirconium	Cl_2	1.0	H_2O	29	-
Zirconium		0.005	H_2O	-	45

D. *Possible Value of Solubility-Depressing Substances for Reducing Caries Incidence*

Several of the substances which can reduce enamel solubility have been tested for their action on hamster caries. A preliminary report by Lazansky[15] described the results of tests on lead fluoride, sodium fluoride, acidulated sodium fluoride and indium, beryllium, and yttrium salts. None of the substances were as effective in reducing the incidence of caries as neutral sodium fluoride. The acidulated fluoride and some of the other salts increased the caries attack rate. McClure[16] presented information indicating that sulfate, iodide, chloride, thiocyanate, and zinc salts had no definite benefit in reducing hamster caries, while salts of copper, iron, and silver were decreasingly effective in the order named. Our findings on solubility effect are in agreement with the results of McClure except for an inversion in the order of activity of copper and iron.

Lead fluoride has been subjected to clinical tests by topical application on human subjects. Galagan and Knutson[17] and Bibby[18] found that caries incidence was not reduced by this salt, while Bibby, Wellock, Roberts, Khenkenberg, Wilkins, and Davenport reported a 30 per cent reduction.[19] Indium nitrate was topically applied without benefit according to one test.[18] Enough of these results are negative to invalidate a generalization that acid-solubility reduction invariably brings about caries reduction.

TABLE II
REDUCTION OF ACID SOLUBILITY OF ENAMEL

SUBSTANCES OR IONS			
MORE ACTIVE THAN 0.1% NaF		LESS ACTIVE THAN 0.1% NaF	
Fluoborate (Pb)	175*	Germanate	41
Gallium	88	Molybdate	40
Yttrium	85	Oxalate*	39
Fluoborate (In)	84	Galliate	36
Fluoride (Pb)*	83	Fluoride (Ag)*	36
Lead	75	Fluoride (Fe)*	33
Indium	73	Fluoborate	32
Fluoride (H)	71	Thallium	31
Fluoride (NH_4H)	70	Aluminum	31
Beryllium	69	Copper*	30
Cerium	69	Ruthenium	29
Fluoride (K_2Ti)	57	Gold*	29
Tripolyphosphate	56	Perrhenate	28
Dysprosium	56	Samarium	28
Pyrophosphate	55	Rhodium	27
Ferric (NH_4)	55	Mercuric	26
Fluoride (Sr)*	54	Lithium	25
Fluoride (Zn)*	54	Silicofluoride	25
Fluoride (Ba)*	51	Cobalt	24
Arsenic	49	Hydrosulphite	24
Thorium	48	Lanthanum	23
Iridium	48	Phytate	23
Rubidium	47	Uranium	24
Vanadium	46	Columbate	20
Zirconium	45	Oleic acid	19
Titanium	43	Didymium	19
Tin	43	Fluoride (Li)*	19
		Fluoride (B)	18
		Fluoride (Ca)	18
		Metavanadate	18
		Zinc	18
		Barium	16
		Caesium	15
		Fluoride (Al)	15
		Strontium	15
		Calcium	11
		Cadmium	11
		Chromium*	10

*In solution in acetate buffer.

The disagreement between the clinical findings with hamsters and the acid-solubility reduction may be attributable in part to insufficient numbers of test animals in some of the groups,[15] but it is improbable that this is also the reason for the variation among tests on human beings. Here the difference between the clinical and the in vitro tests may be traced to variations in the concentration or the pH of the salt solution used, the persistence of the protective layer, and the fact that the in vitro tests were made on powdered enamel whereas the clinical tests are carried out on an intact enamel surface. Indium fluoborate in our acid solubility tests, for example, exerted a decalcifying action at high concentration and protection at low concentration. The protective action of beryllium was easily rinsed off while the fluoride action was not. It seems possible that a refinement in acid-solubility procedure, similar to that which was developed by Brudevold,[14] might give results which paralleled the clinical findings more closely.

It seems to the authors that many of the substances which have been found capable of depressing acid solubility deserve to be tried clinically for their value

in reducing human caries. As an aid in selecting those which are most likely to be beneficial, it would be logical to make a preliminary screening from tests on the surfaces of intact teeth[14] and a second screening with tests on hamsters. After those substances which are more toxic than fluoride had been eliminated, the remainder would be substances which deserve clinical tests with human subjects.

SUMMARY

One hundred forty-seven substances, comprising compounds of fifty-seven elements, have been tested for possible effect on the acid solubility of powdered tooth enamel. The investigations were carried out independently in two laboratories by slightly different procedures. The substances were dissolved in water or acetic acid buffer and placed in contact with enamel powder for a few minutes. Acid solubility of enamel after this treatment was compared with the solubility before treatment.

Twenty-seven compounds were more active in reducing acid solubility than 0.1 per cent NaF, and thirty-eight were less active but definitely effective. Compounds of cations of Groups III and IV of the periodic table were among the most active substances. Several other compounds of fluoride were more active than sodium fluoride in depressing enamel solubility.

The assistance of Miss Ruth Schlotman and Miss Nancy France of the Procter and Gamble Company is acknowledged by one author (RSM) and the aid of Arvid Ek, Michael Buonocore, and Mrs. Irene McGravy is acknowledged by the other (BGB).

REFERENCES

1. Volker, J. F.: *Proc. Soc. Exp. Biol. & Med.* **42**: 725, 1939.
2. Miller, W. D.: *D. Cosmos.* **47**: 913, 1905.
3. Hill, T. J., and Arnold, F. A.: *J. D. Res.* **16**: 23, 1937.
4. Buonocore, M. G., and Bibby, B. G.: *Tufts Dental Outlook* **18**: 15-16, 1944.
5. Buonocore, M. G., and Bibby, B. G.: *J. D. Res.* **24**: 103, 1945.
6. Muhler, J. C., and Van Huysen, G.: *J. D. Res.* **26**: 119, 1947.
7. Rae, J. J., and C. T. Clegg: *J. D. Res.* **27**: 52, 1948.
8. Manly, R. S., and Hodge, H. C.: *J. D. Res.* **18**: 133, 1939.
9. Van Huysen, G., and Muhler, J. C.: *J. D. Res.* **27**: 46, 1948.
10. Lange, N. A.: Handbook of Chemistry, Sandusky, Ohio, 1934, Handbook Publishers Inc.
11. Drea, W. F.: *J. D. Res.* **15**: 403, 1936.
12. Lowater, F., and Murray, M. M.: *Biochem. J.* **31**: 831, 1937.
13. Brown, J. B., and Kitchin, P. C.: Unpublished data.
14. Brudevold, F.: *J. D. Res.* **27**: 320, 1948.
15. Lazansky, J. P.: *J. D. Res.* **26**: 446, 1947.
16. McClure, F. J.: *J. D. Res.* **27**: 34, 1948.
17. Knutson, J. W., and Galagan, D. J.: *Pub. Health Rep.* **62**: 1477, 1947.
18. Bibby, B. G.: *J. D. Res.* **27**: 367, 1948.
19. Bibby, B. G., Wellock, W. D., Roberts, F., Khenkenberg, E., Wilkins, E., and Davenport, R.: *J. D. Res.* **27**: 724, 1948.

EXPERIMENTAL EFFECTS OF THIOURACIL AND SELENIUM ON THE TEETH AND JAWS OF DOGS

JAMES A. ENGLISH, B.S., D.D.S., M.S.*

Naval Medical Research Institute, Bethesda, Md.

PURPOSE AND SCOPE

THE purpose of this paper is to report certain changes in the dentition and bone of the jaws of a litter of dogs treated with two goitrogenic compounds: thiouracil and organic selenium. No other published accounts of experimental studies relating these drugs to oral structures have been found in the literature. The very limited amount of endocrinologic data of any kind pertaining to the dentition has served to make this investigation more interesting and more worth while. It first became apparent that this study might be fruitful when certain gross changes were noted in the teeth of a litter of dogs which had been treated with thiouracil and selenium compounds at the Wyeth Institute of Applied Biochemistry, Philadelphia, Pa., in an experiment directed by Dr. Joseph Seifter.† The investigation reported herein began after the sacrificing of the dogs, when the heads were made available for study. The practical significance of studying these particular drugs is twofold. Both of these compounds fall into a large group of commonly used drugs that have an effect on the thyroid gland, and it is important to know any structural changes that may result from using them. One of the compounds contains the element selenium which is found in sufficient quantities in nature to be of significance in certain areas of the United States and throughout the world.

EXPERIMENTAL PROCEDURE

(Wyeth Inst. Appl. Biochem.) Four mongrel female dogs (Fig. 1), which were litter mates, were used in the experiment. A fifth dog, the runt of the litter, was not used. Three of them were administered drugs, as shown in Table I, and the fourth was kept as a control. They all were fed a normal balanced diet. There was a 21 per cent range of difference in the weight of the dogs at the start of treatment, but one of the smaller dogs was kept as the control. Administration of the drugs was begun at weaning (approximate age, six weeks) and was continued for 25 weeks. At this time the dogs were sacrificed.

The investigation reported herein was performed in the Department of Pathology, Medical School, University of Pennsylvania, in partial fulfillment of the requirements for the degree of Master of Science in Pathology.

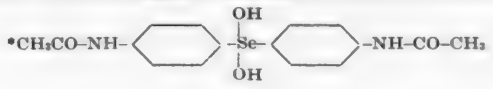
Received for publication July 1, 1948.

*Commander, Dental Corps, United States Navy. The opinions and views contained in this article are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

†Some of the data from this study have been reported by Seifter in *Proc. Soc. Exper. Biol. & Med.* 6: 370, 1947.

TABLE I
SCHEDULE OF TREATMENT

DOG	DRUG USED	DOSAGE PER KG. WT. PER DAY (MG.)	WEIGHT AT BEGINNING OF TREATMENT (KG.)	WEIGHT AT SACRIFICING (KG.)
B2	Organic* Se(OH)2	25	1.5	4.9
B3	Control	—	1.5	10.0
B4	Thiouracil	5	1.9	8.4
B5	Organic* Se(OH)2	5	1.8	11.9



OBSERVATIONS

Description of the Gross Lesions.—No routine clinical oral examinations were made of the dogs during the course of treatment, nor was a record kept of the dates of eruption of the teeth. Fig. 2 shows the heads of the dogs, cleaned of soft tissue, and Table II gives a record of measurements of the jaws and certain teeth.

The jaw of the dog treated with thiouracil and of the one treated with 25 mg. of selenium are smaller than those of the control in proportion to the difference in their body weights (25 per cent and 51 per cent respectively). The dog receiving the smaller doses of selenium shows no significant dimensional changes or gross lesions.

Fig. 1.—Four mongrel dogs that are litter mates are shown at the end of a 25-week experiment. Dog B3 is the control. The other dogs were given daily doses of goitrogens in the following amounts per kilogram of body weight: B5, 5 mg. of selenium; B4, 5 mg. of thiouracil; B2, 25 mg. of selenium. Dogs B2 and B4 are noticeably smaller than the control.

There is no marked change in the size of the crowns of the teeth of the treated dogs. The average coronal height of four teeth (canines and first molars) of both the dog treated with thiouracil and with 25 mg. of selenium

compound is 10 per cent less than those of the control. The average antero-posterior width of the first molars of these same dogs is 4 per cent less than those of the control. These differences are relatively small.

TABLE II
COMPARATIVE MEASUREMENTS (MILLIMETERS)

	B3 CONTROL	B4 THIOURACIL	B2 SELENIUM (25 MG.)	B5 SELENIUM (5 MG.)
Mandible				
Condyle to symphysis	125.5	111.4	105.3	126.0
Height of jaw at carnassial	21.0	17.5	15.0	20.7
Carnassial: height of crown	11.8	9.5	10.0	10.0
Carnassial: a.p. width of crown	19.5	18.2	17.2	18.1
Canine: height of crown	14.9	10.0	12.7	14.2
Total space between premolars	11.3	5.4	0.3	3.0
Maxilla				
Length: symphysis to post-zygoma	125.9	122.0	114.7	132.7
Height of maxilla at carnassial	54.7	40.0	41.0	50.0
Carnassial: height of crown	10.0	9.3	9.0	8.3
Carnassial: a.p. width of crown	17.4	16.8	16.1	16.2
Canine: height of crown	15.0	12.9	12.4	13.0
Total width: zygomatic arch	87.8	81.0	74.0	85.3

There is an alteration in the arrangement of the teeth. The mandibular incisors of B4, the thiouracil-treated dog (Fig. 3), are arranged in a double row. There is not enough space between the canine teeth for them to be in a normal single arch. In Fig. 4 it can be seen that the mandibular canine teeth of B4 are anteriorly displaced, thereby necessitating the irregular position of the incisors. There are incisal areas of abnormal wear on these canine teeth resulting from their altered position in relation to the maxillary teeth. In addition, Dog B2 (treated with 25 mg. of selenium) has two deciduous canine teeth still in its maxilla.

There are several discrete, hypoplastic areas in the maxillary and mandibular canine teeth of the thiouracil-treated dog which are about 1½ mm. in diameter (Fig. 4, B4). They are smooth, shallow, dished out lesions appearing somewhat like small erosions. They occur in the middle portion of the teeth and are about 0.4 mm. in depth. The maxillary third incisors of the same dog show a mottling of the incisal third of the enamel with some loss of tooth structure, resulting in a wedge-shaped tooth. The surface of the teeth in these areas is roughened and markedly stained with brown pigment. There are small, white, opaque areas mottled with gray at the incisal tips of the deciduous canine teeth, the permanent canine teeth, and the third incisor teeth of the dog treated with 25 mg. of selenium (Fig. 4, B2). These mottlings are smooth and the contours of the teeth are normal. The dog treated with 5 mg. of selenium shows a marked surface staining of the incisor teeth with a brown pigment. However, the teeth structure does not seem to be involved (Fig. 4, B5).

The mandibular carnassials (first molars) of the thiouracil-treated dog show a marked hypoplasia of the enamel (Fig. 5, B4). With the exception of a narrow cervical band of enamel, the crowns of these teeth appear to be en-

tirely denuded of enamel. There is a distinct horizontal ridge where the enamel terminates in the cervical region, and a less pronounced ridge across the tips of the anterior and middle cusps. The portion of the tooth that

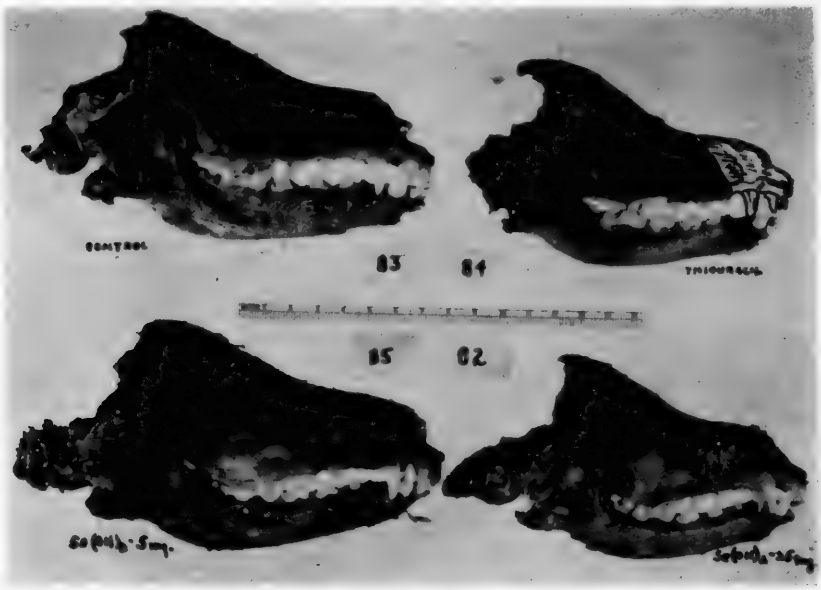


Fig. 2.—The heads of the dogs stripped of skin and muscle show the comparative sizes. The lower borders of the mandible of B4 and B2 are rounded in comparison to the relatively straight inferior border of the mandible of the control. Gross details of the teeth are shown in Figs. 4 and 5. Part of the maxilla of B4 is reconstructed manually.

Fig. 3.—The mandibular incisors of the thiouracil-treated dog (B4) are arranged in a double row. Although there is some void space, the total width of the six incisors is greater than the available room between the canine teeth. It therefore would be impossible for the incisors to be arranged in a normal single arch without changing the position of the canines. Fig. 4 shows that the mandibular canine teeth of B4 are anteriorly displaced.

appears to be uncovered by enamel is a dull yellow in comparison to the gleaming white surface of the carnassial of the control dog (Fig. 5, B3). The maxillary carnassials (fourth premolars) are also hypoplastic, although the area devoid of enamel is not as extensive.

Other gross changes, to be reported elsewhere by Seifter, Pryde, and English, include dwarfing, underdevelopment of the ovaries, fibrotic uteri, immaturity of the vulva, and underdevelopment of the mammae. Fig. 1 shows the difference in size of the dogs just prior to sacrificing.

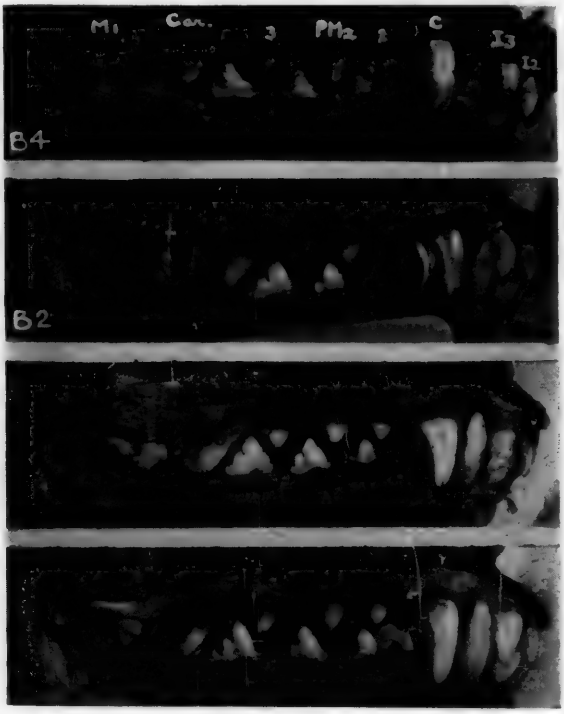


Fig. 4.—(Approx. actual size.) B4—The thiouracil-treated dog shows marked roughening and mottling and some hypoplasia of the maxillary third incisors (I_3) and a localized hypoplasia in the mid-region of the canine (C). The mandibular canine occludes with the lingual portion of the maxillary third incisor (I_3) instead of assuming the normal relationship between the maxillary third incisor and canine. The lesions on the carnassials show to better advantage in Fig. 5.

B2—The selenium-treated dog (25 mg.) has the deciduous canine teeth still in the maxilla, even though the permanent canine teeth have erupted. The incisal tips of the deciduous and permanent canine teeth and of the third incisors of the maxilla have small opaque areas with some mottling. All of the teeth in this dog have a faint orange tinge.

B5—The selenium-treated dog (5 mg.) has a dentition that compares quite favorably with the control, B3. There is more stain on the surface of the anterior teeth but the tooth structure does not seem to be involved. The mandibular carnassial shows a lesion in Fig. 5.

B3—The control dog has teeth that are large, well formed, with glistening white enamel.

Roentgenographic Examination.—The four skulls were split sagittally with a fine jeweler's saw and all halves were exposed over regular 8″ × 10″ x-ray film. Fig. 6 shows prints of the resulting roentgenograms. There are radiolucent areas in the canine teeth of the thiouracil-treated dog (B4) that correspond in position with the hypoplastic gross lesions. These areas have a roentgenographic appearance that is similar to early carious lesions in human teeth. A comparison of the apices of the canine teeth of B4 and B2 with the control (B3) shows a retarding in the closure of the pulp canal. The size of the

teeth of Dogs B4 and B2 seems to be smaller than the control in proportion to the diminution of their heads. The angulation of the mandibular canines of the thiouracil treated dog is more acute than in the control. The deciduous canine teeth remaining in B2 show beginning resorption of their roots, but they are still well fixed in the maxilla. There is an increased density of the bone of the

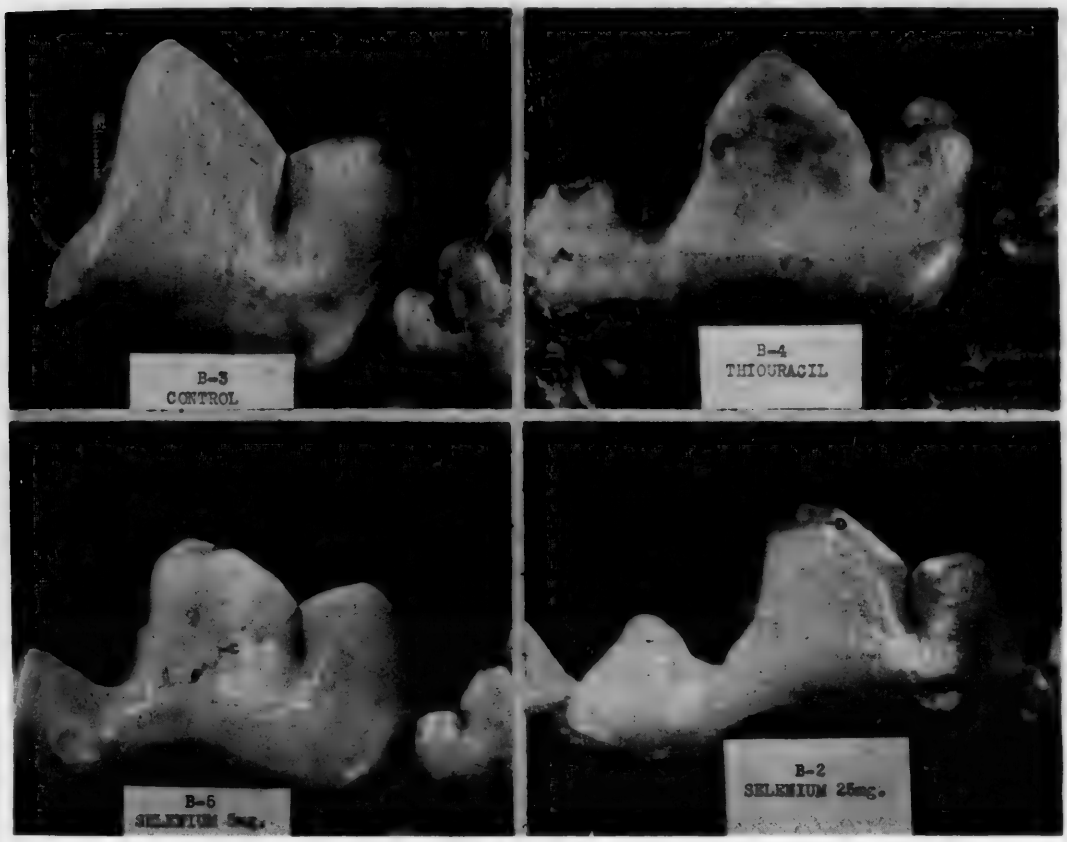


Fig. 5.—Magnification ×5½. The mandibular carnassials offer a good standard for comparing the external appearance of the enamel of the teeth. The enamel of the control (B3) is smooth, glistening, and white. Its contours are even and there are no breaks in the surface or opaque areas. The thiouracil-treated dog (B4) has enamel that is hypoplastic throughout the whole occlusal three-quarters of the crown. The hypoplastic portion has a relatively dull, opaque surface. There is a distinct horizontal ledge (*A*) where the hypoplastic area joins the normal-appearing enamel in the cervical region. There is a broad ridge (*B*) over the anterior and middle cusps where the enamel appears to be thicker. Both selenium-treated dogs, B5 and B2, have enamel that is normal in appearance over most of the surface of the carnassials. However, there are circumscribed areas where the enamel is hypoplastic, indicating local developmental faults. In B5 the main lesion is about 3 mm. in diameter, occurs in the buccal surface of the middle cusp (*C*), and has a base similar to the lesion described in B4. In B2 the carnassial has a hypoplastic area involving the tip of the middle cusp (*D*). It has a roughened surface and is dark in color.

treated dogs, particularly evident in B2 by the increased radiopacity around all of the roots along the lamina dura. There are radiating streaks of dense bone crossing the septa of the alveolar ridges of the treated dogs. By comparison, the bone of the control dog is very regular and homogenous.

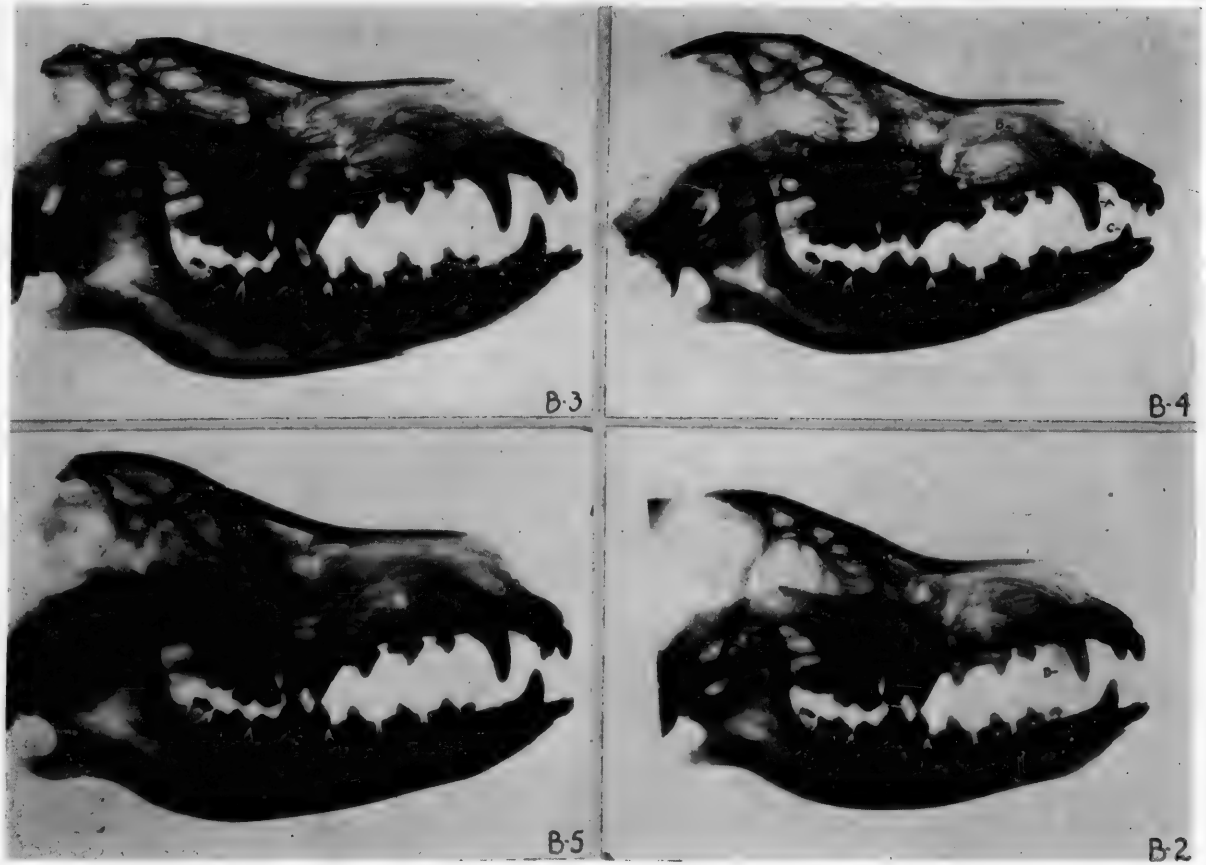


Fig. 6.—Roentgenograms of the four skulls. The skulls were split sagittally and only the right halves are shown here. The other halves are roentgenographically identical to these. B3 is the control dog.

The thiouracil-treated dog (B4) shows radiolucent areas in the canine teeth (*A*) which correspond in location to the enamel hypoplasia. The apical foramen of this tooth (*B*) is wider than that of the canine of the control dog. The mandibular canine (*C*) has a more acute angulation than the same tooth in the control. The size of the teeth is smaller than the control in proportion to the diminution of the size of the skull.

The dog that received a large dose of selenium (B2) shows a retained deciduous canine (*D*). There is an increased density of the bone of this dog (also moderately apparent in B4 and B5) as compared to the control. This is particularly evident by the increased radiopacity around the roots along the lamina dura (*E*) and in streaks of dense bone crossing the septa.

Microscopic Lesions.—The teeth and jaws of each dog were studied histologically by means of ground sections and decalcified sections taken from characteristic regions. The illustrations involving tooth structures are taken from the ground sections, since fully developed enamel is largely dissolved in the decalcification process. The mandibular right carnassials are used as comparative specimens, the sections being made in a buccolingual plane at identical locations through the mesial cusp and root. These sections are unstained (Figs. 7 and 9). Ground sections of the maxillary canine teeth, stained with hematoxylin and eosin, are used to illustrate additional lesions. The sections were all ground to the same thickness as nearly as possible by manual methods. Several small segments of bone and enamel that have been lost in the grinding process can be recognized by the irregularity of the fracture line.

The section of the carnassial tooth of the control animal is the longest (Fig. 7, B3). The enamel surface is even and it is normally graded in thickness from the tip of the tooth to the cemento-enamel junction. It measures 440 microns at the thickest point. The buccal layer of enamel contains alternating bands of dark and light (Schreger's) bands. The dentin in the crown of the tooth photographs black because air in the dentinal tubules causes divergence of the rays of transmitted light. When examined directly in the microscope, the dentin appears normal throughout: the dentinal tubules follow regular "S" curves from the enamel border to the pulp chamber and only a few small, crescent-shaped, interglobular areas may be seen forming a granular layer just under the enamel border in the cervical region. The mandibular supporting bone is composed of large haversian systems that are evenly distributed, and the component lamellae are uniform in thickness. The vascularization is abundant and even in distribution. The mandibular canal is located somewhat buccally to the apical end of the tooth. The general arrangement of the bone in this cross section is such that the tooth has maximum support and the jaw appears to be strong architectually.[1, 2, 3]

The section of the carnassial tooth of the thiouracil-treated dog is 20 per cent shorter than the control (Fig. 7, B4). The enamel of this tooth is extremely hypoplastic through the mid-portion of the crown. The occlusal half of the crown is covered by a cap of enamel that, toward the tip, is about one-half the normal thickness on the buccal side (right side of B4 in Fig. 7) and one-third the normal thickness lingually. Moving gingivally this cap of enamel gradually becomes thinner until just below the middle of the crown it measures only 30 microns (as compared to 350 microns in the control at the same location). At about two thirds the distance from the tip of the cusp to the gingiva there is a sudden thickening of the enamel so as to form a circular ledge around the tooth. From this position gingivally the enamel is normal in thickness and contour. There is some variation in the architectual pattern of the enamel in the hypoplastic areas. In the thinnest portions the enamel rods are indistinct and the outer layer is black when viewed by means of transmitted light (Fig. 9, B4). The enamel rods appear to lean toward the hypoplastic area instead of following the usual course approximately per-

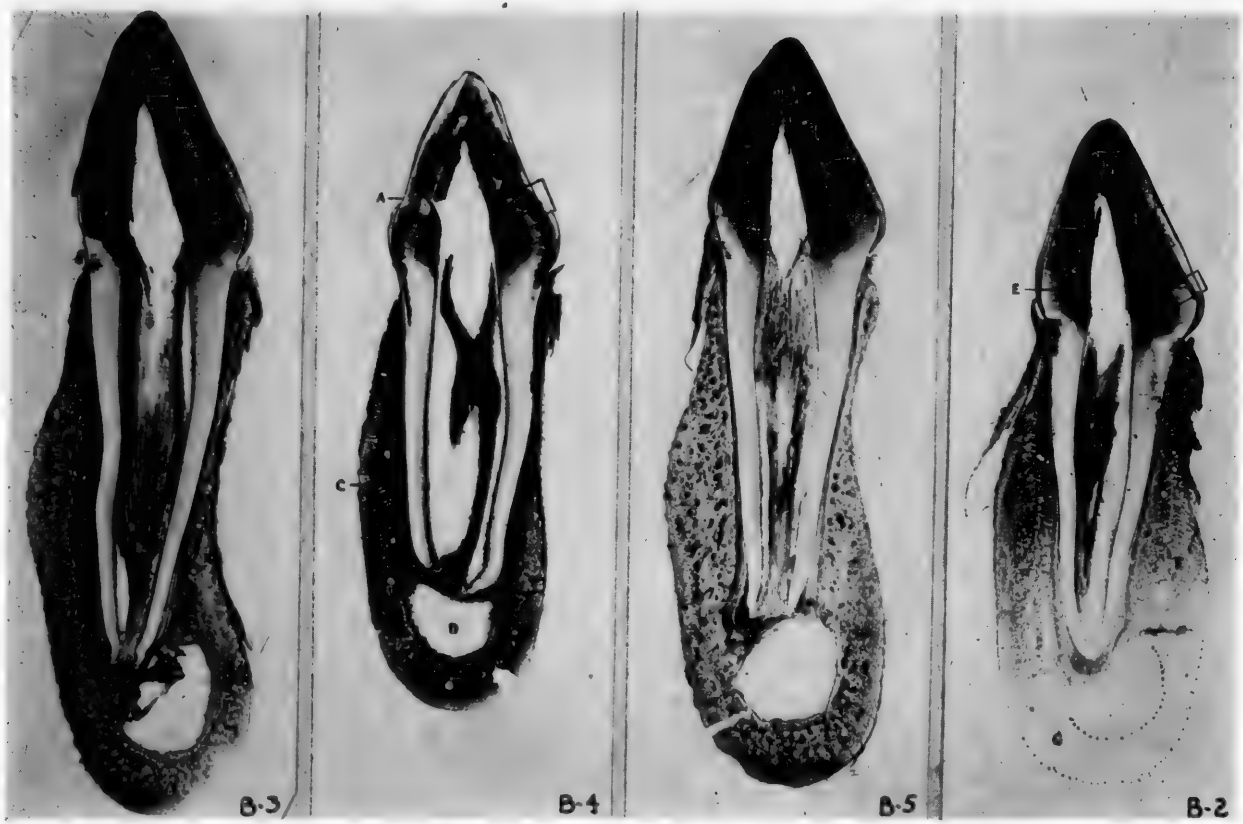


Fig. 7.—Magnification ×6, unstained. Longitudinal ground sections through the mesial cusp and root of the mandibular right carnassials show the variation in size of these teeth and the following main differences:

B3 is the control.

B4, the thiouracil-treated dog shows an extreme degree of hypoplasia of the enamel through the mid-portion of the crown (*A*). The dentin (*B*) transmits light unevenly. The mandibular bone (*C*) is thinner than the control and the haversian systems are smaller and irregular in arrangement as compared to the control. The mandibular canal (*D*) is directly below the apex of the tooth and the architectural arrangement of the bone does not appear to be as strong as the control.

B5, the dog receiving a small dose of selenium, appears normal in this section.

B2, the dog receiving a large dose of selenium, has granular lines running parallel to the dentino-enamel junction (*E*). The mandibular bone (*F*) is thicker than the control; however, there are some large cancellous areas in the buccal and lingual plates. The haversian systems are small and have a different directional arrangement than the control. A portion of this bone (*G*) was lost in grinding.

pendicular to the amelodentinal junction. When viewed directly with the microscope, the enamel rods appear to be frayed toward the outer surface, and frequently the rods blend with each other so as to form masses of minute spheres without a definite pattern. The stained section of the canine tooth of this animal (Fig. 8, B4) includes the margin of a circumscribed lesion that appears to be similar to the extensive lesion described in the carnassial. The enamel becomes very thin, and one cannot be sure that the plaques of malformed black material covering the dentin in the thinnest region actually are enamel. In this dog the coronal dentin (Fig. 7, B4) is irregular in appearance when viewed with transmitted light. There are areas that are black, as described in the control dog, and other areas that are grey or white. These variations in refractive indices of the dentin suggest irregularity in deposition of calcium salts.[1] When viewed directly with the microscope, the dentinal tubules are seen to follow an irregular course in the central part of the tip of the tooth. A granular layer is located in a limited area just beneath the cervical enamel. The mandibular bone supporting the carnassial tooth is composed of haverian systems that are smaller and more irregular in arrangement as compared with the control dog. The vascularization is not as even in distribution nor the lamellae as uniform in thickness as in the control. The mandibular canal is directly below the apex of the tooth. The buccal plate of bone is thin, and the general arrangement of the bone as seen in cross section does not appear to be as strong architectually as in the control dog.

The section of the carnassial tooth of the dog receiving 5 mg. of selenium is 9 per cent smaller than the control (Fig. 7, B5). The enamel is smooth, regularly distributed in thickness, and appears normal in structure. The dentin is similar to that of the control, the only difference being an increase in the number of interglobular areas. These spaces are located in a single band just below the enamel in the cervical region. The characteristics of the mandibular bone and its distribution are similar to that of the control.

The section of the carnassial tooth of the dog receiving 25 mg. of selenium is 18 per cent smaller than the control (Fig. 7, B2). The enamel is normal in appearance, although the incremental lines of Retzius show more plainly than in the control dog. The dentin has granular lines running parallel to the dentino-enamel junction in its central portion. These lines are found both buccally and lingually, and extend from the middle of the crown to about one-third the distance from the cervix to the apex of the tooth. In high power (Fig. 9, B2), these lines are seen to be composed of many small, crescent-shaped interglobular areas, which appear either dark or light depending on the angle of the transmitted light. These areas are not different in appearance from the components of the granular layer described in the control dog, but there are considerably more of them and they are located deep within the dentin. The buccolingual width of the mandibular bone in this animal (Fig. 7, B2) is greater than in any of the other three. However, there are some large cancellous areas in both its labial and lingual plates of bone.

The bone is composed of haversian systems that are smaller than the control and they are arranged in a different directional pattern. They tend to run perpendicular to the long axis of the tooth whereas in the control they tend to run around the tooth. The effect of the size and arrangement of the haversian systems is to give the bone a fine, lacy appearance.

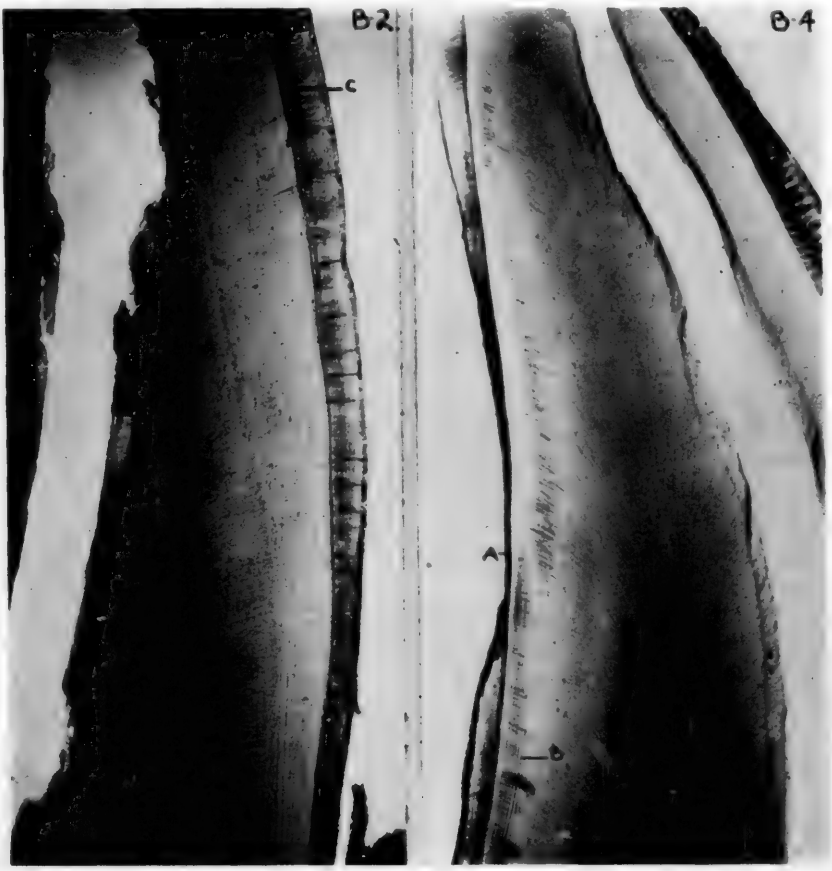


Fig. 8.—Magnification ×16, stained with H. and E. Longitudinal ground sections of the canine teeth show a hypoplastic lesion of the enamel (*A*) and prominent granular layers in the dentin (*B*) in the thiouracil-treated dog (B4). A pronounced incremental line of Retzius (*C*) is apparent in the selenium-treated dog.

The articular condyles of the mandible were studied histologically in order to see what effect the administration of thiouracil and selenium have on this growth center of the lower jaw. The sections are taken in a sagittal plane, thereby including a vertical section through the head and neck of the condyle. The condylar bone was decalcified in formic citrate, embedded in paraffin, and stained in hematoxylin and eosin.

The mandibular condyle of the control dog (Fig. 10, B3) is hemispherical in shape. The articular surface is covered by a thin layer of fibrocartilage

(fibrous capsule) beneath which is an even layer of hyalin cartilage. The trabeculae immediately beneath the cartilage are slender, and many of them contain islands of hyalin cartilage. This is a characteristic of newly formed endochondral bone.[1] Deeper within the head of the condyle the trabeculae become wider and the neck of the condyle is composed of compact bone. In higher magnification (Fig. 11, B3) it is apparent that the cartilage of this animal is inactive, the chondrocytes are fairly small and regular in size, and they are arranged in columns. There is no evidence of rapid calcification of the inner layer of cartilage.

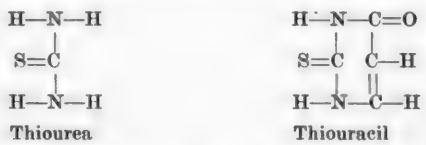
The mandibular condyle of the thiouracil-treated dog (Fig. 10, B4) is much narrower than that of the control animal, and the articular surface is irregular at the peripheral ends. The fibrous capsule is uneven in thickness. It is thin in the center of the articular arc and thick and folded at each end. There is a layer of hyalin cartilage in the middle of the articular surface of the condyle but it abruptly ends about one-quarter of the distance from each end as seen in cross section. The trabeculae in the head of the condyle are not radially arranged as in the control dog. This haphazard arrangement of the trabeculae appears to offer less support to the outer compact layers of bone of the condyle. The bone of the neck looks dense. In higher magnification (Fig. 11, B4) it is apparent that the condylar cartilage of this dog is relatively inactive. The cartilage and bone are histologically quite similar to that of the control.

The mandibular condyle of the dog treated with 5 mg. of selenium (Fig. 10, B5) is a little wider than that of the control but it is flattened on its articular surface. The fibrous capsule is even in thickness. A band of hyalin cartilage extends over proportionally as long an arc as in the control, although it is greater in width in the central half of the arc. Even in low magnification the cartilage appears to be active in this region and the growing chondrocytes are pale due to vacuolization. Compared with the control, Dog B5 has a greater number of bony trabeculae in the primary layer beneath the hyalin cartilage, as well as in the head of the condyle. There are islands of cartilage in the trabeculae to a greater depth within the condyle of this dog than in the control. The trabeculae are relatively thin and the bone of the neck of the condyle is interrupted by large marrow spaces. In higher magnification (Fig. 11, B5) it is evident that there is moderate proliferation and growth of the chondrocytes in the band of hyalin cartilage. The cells are irregular in size, they are disorderly arranged, and degeneration of the large, edematous cells is apparent. The inner zone of the cartilage is becoming calcified. Growth of endochondral bone is not occurring at the extreme ends of the articular arc. When examined under the microscope, signs of periosteal bone growth may be observed on the inferior surface of the neck of the condyle. Osteoblasts are lined up all along the trabeculae on this side. On the opposite side of the neck a number of osteoclasts may be observed reabsorbing bone, probably for the purpose of remodeling the neck in conjunction with its growth.

The mandibular condyle of the dog treated with 25 mg. of selenium (Fig. 10, B2) is wider than that of the control, but it is considerably flattened on the articular surface.* There is a marked proliferation of chondrocytes in the outer layer of the articular cartilage and the resulting growth has produced a wide band of hyalin cartilage. At the inferior border the cartilage bulges into a curved lip that is strikingly similar to the "lipping" process sometimes observed in osteo-arthritis. The bone of the head and neck of the condyle is heavily laminated and dense. In higher magnification (Fig. 11, B2) zones of proliferation, growth, and degeneration of cartilage are seen progressing from the fibrocartilage toward the bone. The chondrocytes are not arranged in orderly columns. Bands of basic staining tissue run irregularly into the cartilage from the trabecular surface and mark the areas of calcification of cartilage which will form the basis for new bony trabeculae. Proliferating connective tissue can be seen invading the degenerated cartilage between the spicules of cartilage that have become calcified. In addition to this very active endochondral bone formation, there is a marked periosteal development of bone along the inferior border of the neck of the condyle. A wide band of new bone may be seen here with osteoblasts lined up in an even row all along the surface. On the opposite side of the neck the osteoclasts are equally active in the resorption process.

DISCUSSION

The Pharmacologic and Chemical Nature of Thiouracil.—Thiouracil is one of a group of chemically related compounds, containing the HN—CS—NH structure, which will produce hyperplasia of the thyroid gland.[4, 5] The basic compound, thiourea, has a simpler structure, as can be seen in the formulae:



Thiourea Thiouracil

There are many other compounds that have a goitrogenic effect on the thyroid gland, some being directly related to the above compounds (as 6-n-propylthiouracil[6]) and others having an entirely different structure (as the sulfonamides[4] and selenium compounds[7]). Although these drugs have the effect of enlarging the thyroid gland in a manner that is grossly and histologically comparable to hyperplastic or toxic goiter, the symptoms produced are not those of hyperthyroidism but rather the symptoms of blockage of synthesis of thyroxin, lowered metabolic rate, retarded development and growth, decreased food intake, and slowing of reactions.[4, 5, 8] It is believed that the growth of the thyroid gland is actually an attempt on the part of the body to produce sufficient thyroid hormone, the stimulation coming by way of the anterior pituitary gland, which becomes less inhibited as the amount

*A thin section of cartilage was accidentally trimmed off of this articular surface when the jaw was disarticulated. Dotted lines reconstruct approximate former margin of condyle.

of thyroxin decreases. The thyroxin of the thyroid gland has an inhibiting action on the anterior pituitary gland, whereas the thyrotropic hormone of the pituitary gland stimulates growth and activity of the thyroid gland.[9] The primary action of the thiouracil and similar compounds is an interference in the production of thyroxin within the thyroid gland itself, either by blocking iodine utilization, interfering with enzyme reactions, or by serving as a chemical obstruction to synthetization.[5, 8, 9, 10] That the action of thyroxin itself is not interfered with may be proven by giving the synthetic hormone (thyroxin) at the same time as the drug (thiouracil) is administered, and noting that under these conditions metabolism is normal and the thyroid gland is not stimulated. Hyperplasia of the thyroid gland does not occur in animals experimentally treated with thiouracil if the pituitary gland is removed; however, the administration of iodine does not prevent hyperplasia.[4, 8, 11] The hyperplasia therefore appears to be compensatory to the failure of the thyroid gland to synthesize thyroid hormone.

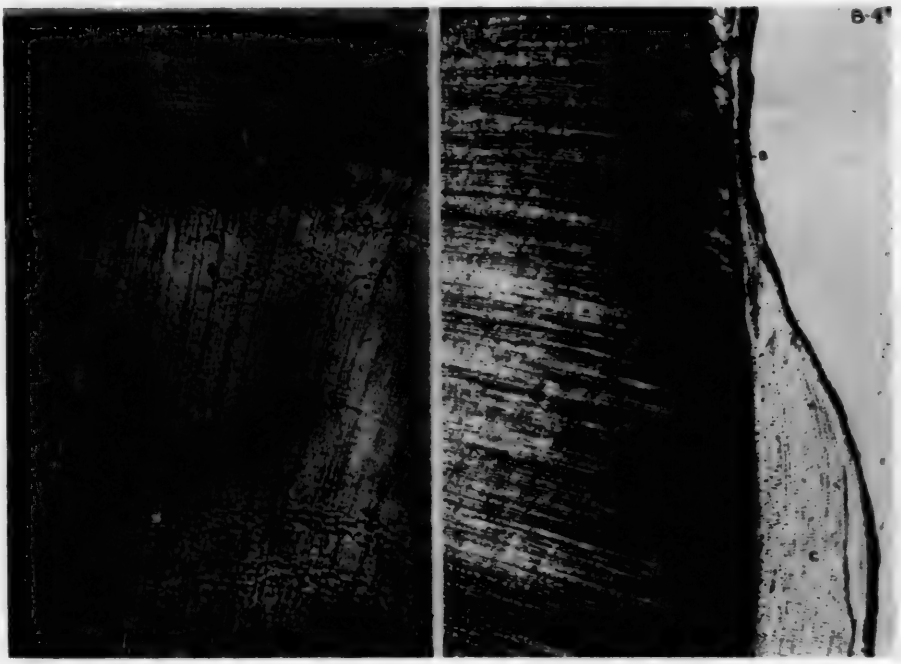


Fig. 9.—Magnification ×140. Enlargements of areas blocked out in Fig. 7 show interglobular spaces (*A*) in the dentin of the selenium-treated dog (B2) and hypoplasia of the enamel (*B*) in the thiouracil-treated dog (B4). The wide portion of the enamel (*C*) is normal (the dark line is an artifact).

The Therapeutic Use of Thiouracil.—It is well established that the administration of thiouracil and related drugs in the treatment of human hyperthyroidism results in clinical improvement in from two days to several weeks. The effect of the drug is evident only during its administration unless the therapy is continued for about nine months. If this is done, there is an ap-

parently permanent suppression of the symptoms of hyperthyroidism in 60 to 80 per cent of recorded cases. In certain cases, such as nodular goiter or large disfiguring thyroid hyperplasia, in which surgery is the preferred treatment, preoperative therapy with thiouracil is of value in reducing the toxic condition.[8, 11, 12]

Toxic Effects Resulting From the Clinical Use of Thiouracil.—A number of references are to be found in current literature pertaining to the toxic effects of thiouracil when used clinically. It is noted that agranulocytosis is the complication most to be feared and that it occurs in about 0.8 per cent of the cases.[13] There have been a few fatalities resulting from this complication. Agranulocytosis may be due to depression of the bone marrow on a toxic chemical basis or as an allergic response. Other reactions noted by many observers are fever, jaundice, swelling of the submaxillary glands and lymph nodes, cutaneous reactions, joint and muscle pain, and gastric disturbance.[8, 13, 14, 15]

A myxedematous picture appears if there is an extensive use of these goitrogenic drugs,[5, 8] and in the case of young rats there is extreme retarding of growth with cretinism resulting from continued administration of thiouracil.[4, 16] Atrophy of the testicles and the adrenal cortex have been observed experimentally in cats as a result of thiouracil feeding.[17]

The Nature and Occurrence of Selenium.—Selenium is a nonmetallic element that is poisonous to most animals if consumed in a concentration exceeding about ten parts per million for a period of ten weeks. It has a wide distribution in the soil of arid regions throughout the world and occurs in appreciable quantities in Wyoming and South Dakota. It is the only element known to be absorbed by plants in sufficient quantities to produce poisoning in the animals that eat them. Grain and other food grown in selenium areas are a potential danger to livestock, and cattle raised in these areas frequently develop a disease known as "alkali disease." The effect on livestock is emaciation, stunted growth, stiff gait, loss of hair, atrophy or actual loss of hoofs, a type of liver cirrhosis, and impairment of reproduction. It is believed that human inhabitants of these areas may also suffer a minor involvement of the gastrointestinal tract, liver, nerves, fingernails, and teeth from consuming local produce and meat from contaminated cattle. Human selenium poisoning may result from exposure in certain industrial plants (metal smelting, ceramics, paint manufacture).[11, 18, 19]

Fig. 10.—Magnification ×14, decalcified section, stained with H. and E. Sagittal sections through the middle of the mandibular condyles show variations in size and shape of the condyles and the following structural differences:

Section B3 is from the control.

Section B4, from the thiouracil-treated dog, shows irregularity of the fibrocartilage (*A*), termination of the hyalin cartilage prior to reaching the edge of the condyle (*B*), and poor arrangement of the trabeculae (*C*). The void space in the head of the condyle is an artifact.

Section B5, from the dog that received a small dose of selenium, shows thickening and moderate growth of the hyalin cartilage (*D*), relatively thin trabeculae in the head of the condyle (*E*), and large marrow spaces in the neck of the condyle (*F*).

Section B2, from the dog that received a large dose of selenium shows marked proliferation of chondrocytes in a wide band of hyalin cartilage (*G*) that protrudes in a lip at the inferior border. There is a wide band of periosteal bone formation (*H*) along the inferior margin of the neck. The bone of the head and neck of the condyle (*I*) is heavily laminated and dense. The void space in the center of the condyle is an artifact.

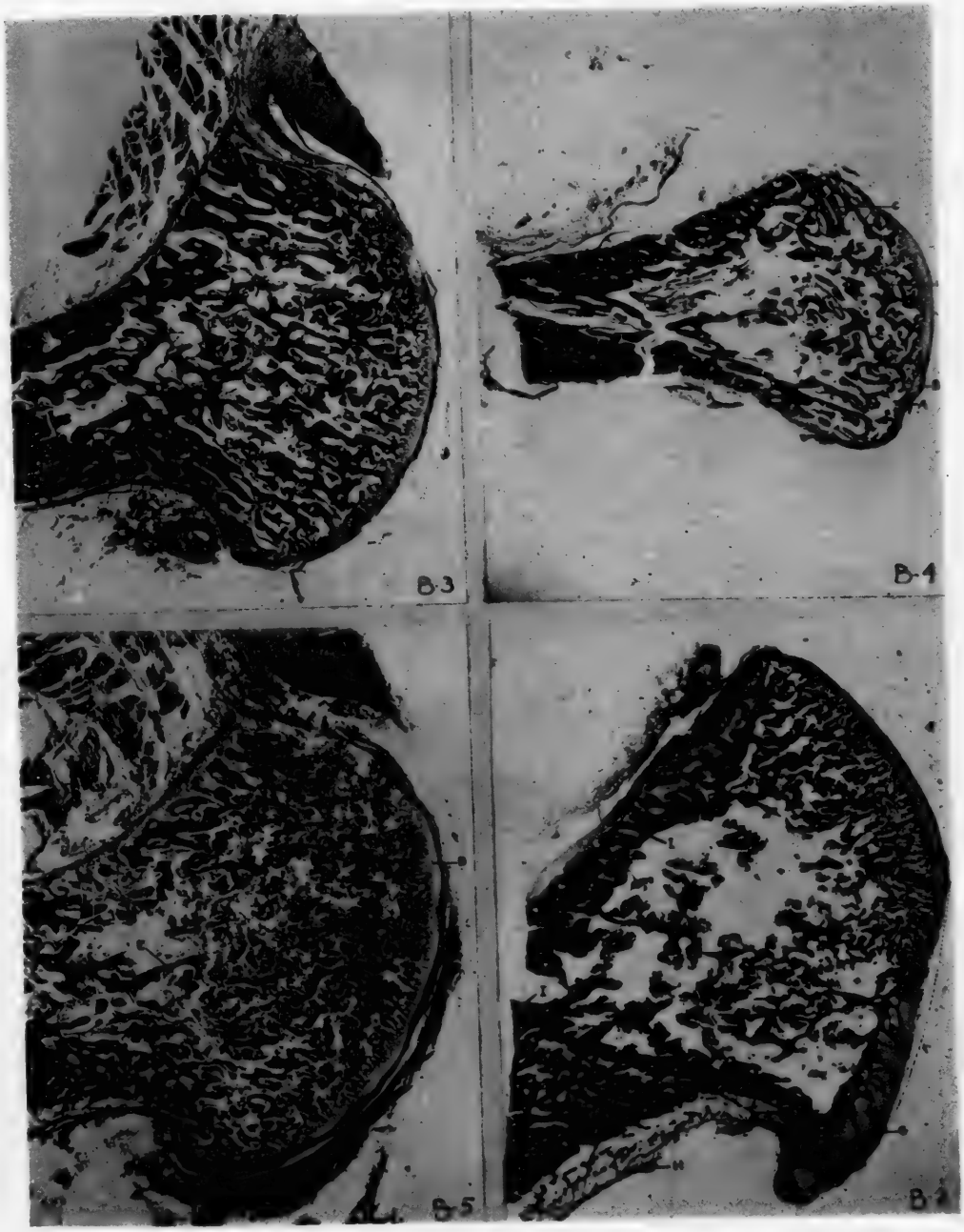


Fig. 10.

(For legend, see opposite page.)

The selenium compound used in these experiments has a chemical structure analogous to that of the sulfa drugs and was shown to be antithyroid when given experimentally to rats.[7] The antithyroid action was specific for this particular selenium compound. A similar effect was not noted when several other selenium compounds, organic and inorganic, were used. The experiment with the dogs was concluded because the dog receiving 25 mg. of selenium compound began to exhibit early signs of selenium poisoning.

It has been experimentally shown that selenium causes a restricted food intake, a decrease in the rate of growth, and a nodular hyperplasia of the liver similar to cirrhosis.[7, 11, 18, 20] The mechanism of action has not been thoroughly studied, but it has been suggested that there is an inactivation of enzymes concerned with metabolic processes.[19]

Endocrine and Metabolic Changes Resulting From Decrease in Thyroid Hormone.—Since thiouracil inhibits the synthesis of thyroid hormone, the endocrinological effects are similar to those of hypothyroidism or thyroidectomy. In experimental thyroidectomy the number of eosinophilic cells in the hypophysis is greatly reduced, although the anterior lobe is enlarged by increase in number of basophils. Inasmuch as the eosinophilic cells of the pituitary gland produce the growth hormone, it is theoretically possible, though not proved, that a secondary pituitary dysfunction may result from a lack of thyroid hormone. The pituitary is restored to normal, histologically and functionally, by administering thyroid extract to the animal. The diminished stature of the thiouracil-treated dog may possibly be due in part to changes in the pituitary gland, but more important may be the direct effects of the decreased cellular metabolism which result from thyroid deficiency, causing stunting of growth. Certain changes which are observed in cretinism may be expected in varying degrees in thyroxin deficiency induced by thiouracil. Among these changes are failure of cartilage to become ossified in various parts of the body, and retarded development and eruption of teeth. The size of the teeth may not be affected, and since the body of the maxilla and mandible are underdeveloped, the teeth may seem overly large.[3] In many cases the teeth erupt in faulty order and in unusual positions. There is alteration in the structure of the teeth and the incisors may be small, conical in shape, and widely separated. Their surfaces may be striated, either longitudinally or transversely. The canines may be misshaped so as to resemble incisors.[21] In thyroidectomized animals a definite line of hypoplasia of the enamel may be seen corresponding to the date of thyroidectomy.[22] It seems to be significant that the treated dogs of this experiment

Fig. 11.—Magnification ×35. Enlargements from Fig. 10. In B3, the control, relatively inactive cartilage borders the articular surface, the trabeculae are radially arranged, and the bone is dense.

In B4, from a thiouracil-treated dog, the cartilage is slightly more active than the control, the trabeculae are not so well arranged, and islands of cartilage make up a large portion of the trabeculae.

In B5, from a selenium-treated dog (5 mg.), the articular cartilage is moderately active and the trabeculae are thinner than in the control.

In B2, from a selenium-treated dog (25 mg.) the articular cartilage is very wide and stages of proliferation, growth, degeneration, and calcification of chondrocytes may be identified. Periosteal bone formation may be seen along the neck of the condyle. The bone is heavily laminated and dense.

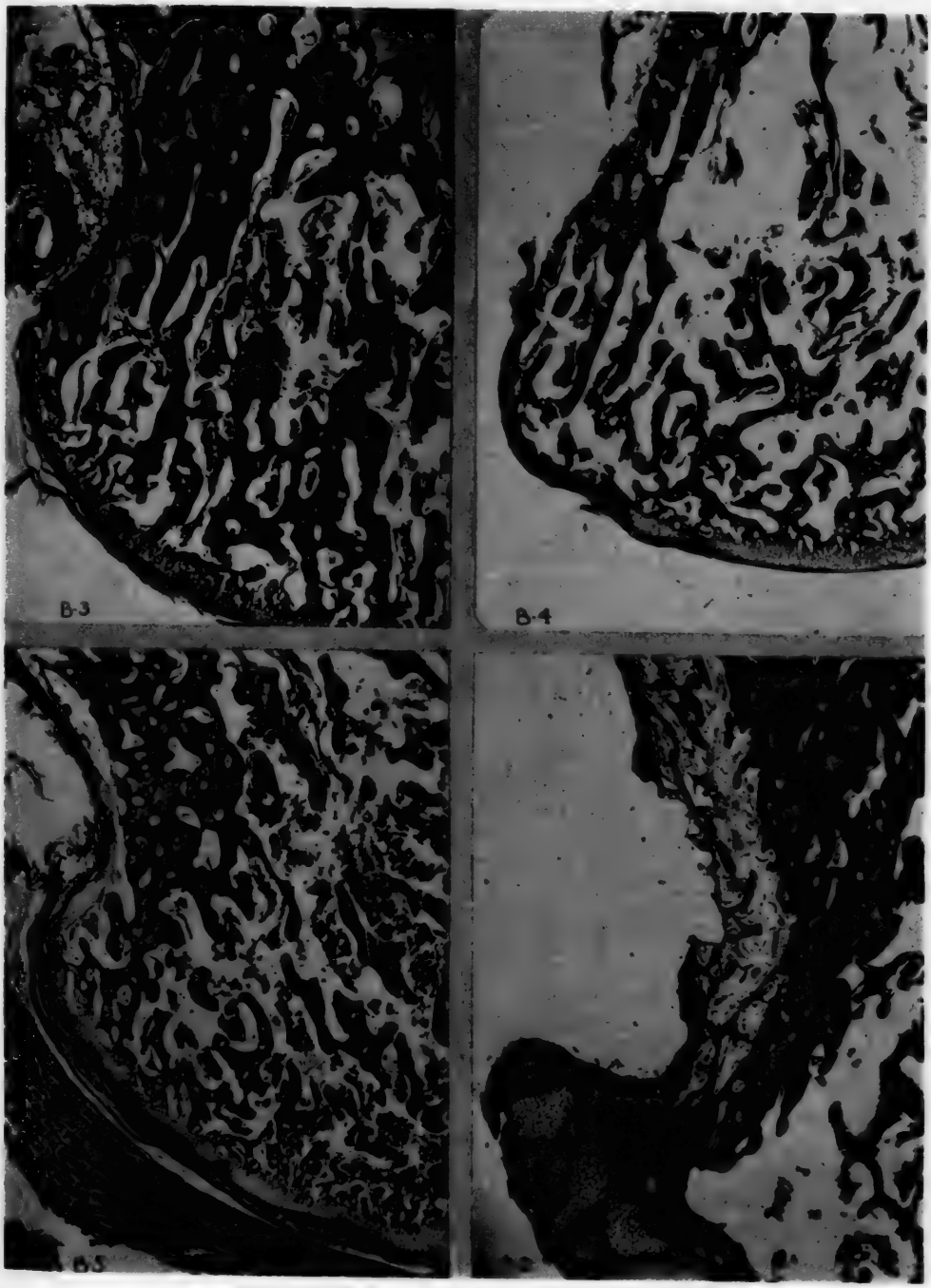


Fig. 11.

(For legend, see opposite page.)

should show comparable lesions, namely, faulty occlusion of teeth, wide separations, surface mottlings of the enamel with alteration in structure and shape, and the prominent line of hypoplasia described in the carnassials.

It is possible to draw an interesting hypothesis in regard to the distribution of the enamel in the carnassial tooth of the dog treated with thiouracil (Fig. 7, B4). (Similar lesions were evident in the other three carnassials which were not sectioned.) It is noted that the *tip* of this tooth has a layer of enamel, and also that the *cervical* part of the crown is substantially covered by enamel.

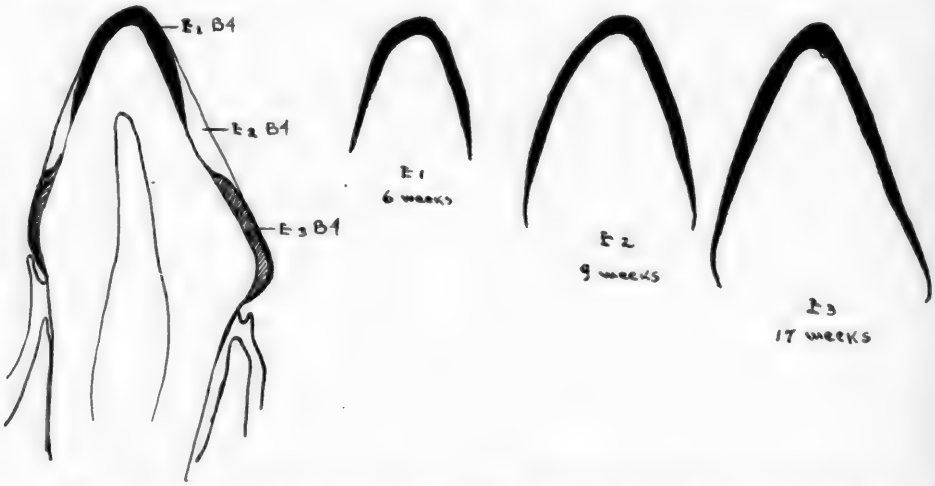


Fig. 12.—Diagram showing manner in which enamel is laid down on the crown of a normal tooth, and explaining mechanism which may have been involved in formation of hypoplastic enamel lesion in thiouracil-treated dog B4.

E_1 represents amount of enamel that normally covers tip of dog's mandibular carnassial tooth at age of six weeks. $E_1B_4^1$ shows this enamel cap on carnassial of thiouracil-treated dog.

As dogs grow older, additional ameloblasts mature toward gingiva, and more enamel is formed. E_2 represents amount of enamel normally present at end of nine weeks. $E_2B_4^1$ shows area of hypoplastic enamel in carnassial of the thiouracil-treated dog.

At age of 17 weeks some enamel covers whole crown of tooth, as shown by E_3. $E_3B_4^1$ represents ring of enamel that covered cervical region of crown of thiouracil-treated dog. It appears that there was a three to five weeks period, beginning with initiation of thiouracil treatment, in which ameloblasts of dog B4 did not function.

The middle portion of the crown, however, shows a definite hypoplasia. At the time treatment was begun in this dog, when it was six weeks old, this tooth was still in the processes of developing. At the age of six weeks in a normal dog, there is a cap of enamel on the tip of the tooth, which extends down over about a third of the crown in a gradually thinning layer (Fig. 12, E1).[2] Normally during the next three or four weeks additional ameloblasts mature toward the cervical region and additional layers of enamel are laid down, so that by an age of nine weeks the enamel is found nearly to the cervical region (Fig. 12, E2). By the time seventeen weeks have passed, some enamel covers the whole crown (Fig. 12, E3). It appears that in the thiouracil-treated dog there was a three to five weeks' period (following the beginning of administration of thiouracil) during which the ameloblasts were inhibited from their normal function of forming enamel. Then a tolerance to this drug may gradually

have been acquired, so that by the time the final deposition of enamel occurred over the cervical region, its development was relatively normal both in thickness and structure. Inasmuch as the dosage of thiouracil was regulated according to body weight, it is hard to interpret this restoration of function of the ameloblasts in any other way than as a tolerance or a successful elimination of the drug.

The disturbance in function of the ameloblasts which resulted in enamel hypoplasia in the thiouracil-treated dog may have been due to inadequate nutrition. The administration of thiouracil reduces metabolism[8] by way of the thyroid gland. This, in turn, could reduce the nutritional supply of the enamel-forming cells. Other studies of different types of enamel hypoplasia have shown that whatever the cause of the hypoplasia, the basic mechanisms involved are somewhat similar. In avitaminosis A, for example, there is atrophy of the enamel-forming cells,[23] which undoubtedly accounts for enamel hypoplasia. Lack of vitamin D during the age of tooth development may also cause hypoplasia.[2] The effect of bacterial invasion, as exemplified by congenital syphilis, causes characteristic enamel lesions which may be due to atrophy of the ameloblasts on a nutritional basis.[24, 25] An enamel hypoplasia of human deciduous teeth resulting from a birth injury has been described.[26] In this case, the ameloblasts, which were active at the time of injury at birth, ceased functioning. Subsequently a cervical ledge of normal enamel was formed by the cells of the enamel organ, which were undifferentiated at the time of the birth injury. The lesions in this case were believed to have been due primarily to a prolonged state of malnutrition. In each of the examples cited above, there was a disturbance in function of the ameloblasts with a resulting failure in enamel formation.

Comparable lesions in the dentin may be explained in somewhat the same way. That is, the odontoblasts may be affected by changes in metabolism, by malnutrition, by endocrine gland dysfunction, or by various disease processes, thereby altering their production of dentin matrix. However, the dentin is much less reactive than enamel.[26]

The dog treated with a large dose of selenium retained its maxillary deciduous canine teeth at the time of sacrificing (220 days). These teeth normally are shed at an age of 160 days. It was reported by Mellanby[2] "that the (deciduous) canines and third molars often remain in the jaw long after the eruption of the permanent teeth when the puppies are fed on certain defective diets." She also reported that the formation of interglobular areas may be due to avitaminosis D. Inasmuch as selenium causes a diminished appetite,[18, 20] a possible dietary deficiency must be considered along with metabolic interference as a related factor in the delayed exfoliation of teeth and the occurrence of interglobular dentin. It has been shown that starvation can cause certain gross and microscopic changes similar to those observed in feeding goitrogens.[27] The internal mechanisms which are involved in starvation and in feeding goitrogens may be the same. Although the refusal of animals to eat diets adulterated by goitrogens complicates experimental procedures, human clinical studies with related compounds show that more than simple starvation is involved.

A recent study of the effect of thyroidectomy on the mandibular joint of rats[28] reveals that with thyroidectomy the cartilage of the condylar head remains wide for long periods, there is disorder in the columns of chondrocytes, and there is increased density of the bone in the head and neck of the condyle. The changes observed in the condyles of the treated dogs of this experiment are not opposed to the above findings. The best parallel is noted in the selenium-treated dogs. The mechanism of interference with metabolism in the case of selenium is not as well established as in the case of thiouracil. That there is an antagonism between selenium and protein metabolism is quite certain, and it is probably due to a differential action of selenium compounds on enzymes concerned with metabolic processes.[19]

Some of the skeletal changes noted in hypopituitarism and in hypothyroidism may be linked with the atrophy of the gonads, which is common to both. Postponement of skeletal maturity and suppression of secondary sex characteristics occur in hypogonadism. However, overgrowth of the skeleton, rather than dwarfing, is the usual result. Epiphyseal cartilage continues proliferation beyond the time of normal closure.[3] The skeletal changes and failure of development of the mammae, uterus, and vulva in the treated dogs of this experiment, as previously noted, may well be due to secondary atrophy of the gonads.

The fact that the dogs used in this experiment were not full-bred animals diminishes the significance of the growth factors reported. It has been shown that the size of the offspring of mated full-bred dogs of different strains is impossible to predict with accuracy, so in the case of mongrels this factor would be even more indefinite.

Thiouracil will not often be administered at an age when it can influence skeletal or dental growth, because thyrotoxicosis occurs most commonly between the ages of 18 and 50.[29] Since neither hypopituitarism nor hypothyroidism cause gross changes in the adult skeleton,[3] administration of thiouracil to adults would not be expected to cause skeletal or dental changes. However, other drugs which cause thyroid hyperplasia by interference with thyroxin formation, for example the sulfonamides, may be administered to growing children. Likewise selenium, which is a dietary problem in certain localities, is ingested at all ages, and is known to have definite effects on growth.[30]

SUMMARY

This is a report of certain changes observed in the dentition and jaws of a litter of dogs treated with goitrogenic compounds: thiouracil and selenium. The important lesions described are:

1. Hypoplasia of the enamel in a thiouracil-treated dog.

2. Structural defects of the dentin in a dog treated with a large dosage of selenium.

3. Structural changes in the supporting bone of the mandible in both a thiouracil-treated dog and a dog receiving a large dose of selenium.

4. Marked variation in the size and shape of the mandibular condyles of all treated dogs, with histological indications of an untimely continuation of bone formation in the condyles of the selenium-treated dogs.

There are tabulations of the schedule of treatment given prior to the initiation of this study, and of the measurements taken of the teeth and jaws at the termination of the experiment. Photographic and photomicrographic illustrations are shown of gross lesions and of characteristic histologic sections of the teeth, jaws, and condyles. A roentgenographic study of the four specimens is included.

A brief review of the pharmacology, therapeutics, and toxicology of thiouracil and selenium is given. Also a brief review is presented of current literature dealing with the endocrine and metaboiic changes occurring in experimental or therapeutic administration of these compounds.

Finally, correlations are made between the findings in this experiment and the various data reported from the literature. It is shown that the gross lesions noted in these dogs are not unlike those found experimentally in thyroidectomy or in the human cretin. Histologically the sections of the dogs' teeth show lesions that are similar to those found in various nutritional disorders, or in diseases in which the underlying mechanism causing structural change have a metabolic or nutritional basis. Sections of the mandibular bone and of the condyles of these dogs have structural modifications which correspond with changes found in thyroidectomy, and hypophysectomy, or in the gonadal atrophy which may result from either of the former endocrine dysfunctions.

Comments are made pertaining to limitations in the significance of the results of this experiment and their interpretations. It is emphasized that this study is based on experimental findings in a single litter of four mongrel dogs. The results, therefore, must have their greatest value in stimulating and orientating future investigations along similar lines.

Advice and assistance was generously given in this study, and in preparation of this paper, by Dr. Morton McCutcheon and other members of the Department of Pathology and of the School of Dentistry, University of Pennsylvania.

REFERENCES

1. Orban, B.: Oral Histology and Embryology, St. Louis, 1944, The C. V. Mosby Company, pp. 53, 100, 190, 310.
2. Mellanby, M.: Diet and the Teeth: An Experimental Study Part III, Medical Research Council, Special Report Series, No. 191, London, 1934, His Majesty's Stationery Office, pp. 38-53.
3. Weinmann, J. P., and Sicher, H.: Bone and Bones, St. Louis, 1947, The C. V. Mosby Company, pp. 18, 45, 186.
4. Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R.: Action of Certain Sulfonamides and of Thiourea Upon the Function of the Thyroid Gland of the Rat, *Endocrinology* **32**: 210-228, 1943.
5. Newman, E. V.: Thiouracil and Hyperthyroidism, *M. Clin. North America* **29**: 302-322, 1945.
6. Astwood, E. B., and Vander Laan, W. P.: Thiouracil Derivatives of Greater Activity for the Treatment of Hyperthyroidism, *J. Clin. Endocrinol.* **5**: 424-430, 1945.
7. Seifter, J., Ehrich, W. E., Hudyma, G., and Mueller, G.: Thyroid Adenomas in Rats Receiving Selenium, *Science* **103**: 762, 1946.
8. Haines, S. F., and Keating, F. R., Jr.: Clinical Use of Thiouracil, *M. Clin. North America* **30**: 845-854, 1946.

9. Rawson, R. W., Evans, R. D., Means, J. H., Peacock, W. C., Lerman, J., and Cortell, E.: Thiouracil Action Upon Thyroid in Graves' Disease, *J. Clin. Endocrinol.* **4**: 1-11, 1944.
10. DuBois, K. P., and Erway, W. F.: Mechanism of Action of Thiourea and Related Compounds; Inhibition of Oxidative Enzymes and Oxidations Catalyzed by Copper, *J. Biol. Chem.* **165**: 711-721, 1946.
11. Sollmann, T.: A Manual of Pharmacology, ed. 7, Philadephia, 1948, W. B. Saunders Co., p. 844.
12. Beirwaltes, W. H., and Sturgis, C. C.: Remissions in Thyrotoxicosis After Discontinuing Thiouracil, *J. A. M. A.* **131**: 735-738, 1946.
13. Fowler, E. F.: The Treatment of Hyperthyroidism With Thiouracil, With Particular Reference to Toxic Reactions, *Surg., Gynec. & Obst.* **83**: 313-323, 1946.
14. Gargell, S. L., and Lesses, M. F.: Toxic Reactions to Thiouracil, *J. A. M. A.* **127**: 890-898, 1945.
15. Beirwaltes, W. H., and Sturgis, C. C.: Complications Following the Administration of Thiouracil, *Am. J. Med. Science* **212**: 513-522, 1946.
16. Hughes, A. M.: Cretinism in Rats Induced by Thiouracil, *Endocrinology* **34**: 69-76, 1944.
17. McClosky, W. T., Lillie, R. D., and Smith, M. I.: The Chronic Toxicity and Pathology of Thiouracil in Cats, *J. Pharmacol. & Exper. Therap.* **89**: 125-130, 1947.
18. Fitzhugh, O. G., Nelson, A. A., and Bliss, C. I.: The Chronic Oral Toxicity of Selenium, *J. Pharmacol. & Exper. Therap.* **80**: 289-299, 1944.
19. Smith, M. I.: Chronic Endemic Selenium Poisoning, *J. A. M. A.* **116**: 562-567, 1941.
20. Rhian, M., and Moxon, A. L.: Chronic Selenium Poisoning in Dogs and Its Prevention by Arsenic, *J. Pharmacol. & Exper. Therap.* **78**: 249-263, 1943.
21. Ehrich, William E.: Pathology for Students and Practitioners of Dentistry, Philadelphia, 1941, Lea & Febiger, pp. 33-35.
22. Ziskin, D. E., and Applebaum, E.: Effects of Thyroidectomy and Thyroid Stimulation on Growing Permanent Dentition of Rhesus Monkeys, *J. D. Res.* **20**: 21-27, 1941.
23. Boyle, P. E.: Manifestations of Vitamin A Deficiency in a Human Tooth-Germ, *J. D. Res.* **13**: 39-50, 1933.
24. Karnosh, L. J.: Histopathology of Syphilitic Hypoplasia of the Teeth, *Arch. Dermat. & Syph.* **13**: 25-42, 1926.
25. Romer, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Henke and Lubarsch, Berlin, 1928, Vol. IV/2, sec. D, pp. 162-184.
26. Schour, I., and Kronfeld, R.: Tooth Ring Analysis, *Arch. Path.* **26**: 471-490, 1938.
27. Seifter, J., and Ehrich, W. E.: Goitrogenic Compounds: Pharmacological and Pathological Effects, *J. Pharmacol. Exper. Therap.* **92**: 303-314, 1948.
28. Becks, H., Collins, D. A., Asling, C. W., Scow, R. O. Simpson, M. E., and Evans, H. M.: Growth and Transformation of the Mandibular Joint in the Rat. IV. The Effects of Thyroidectomy at Birth, *Oral Surg., Oral Med. and Oral Path.* **1**: 315-318, 1948.
29. Williams, R. H.: Use of Thiouracil in the Treatment of Thyrotoxicosis, *M. Clin. of North America* **28**: 1043-1053, 1944.
30. Franke, K. W., and Potter, V. R.: The Effect of Selenium Containing Foodstuffs on Growth and Reproduction of Rats at Various Ages, *J. Nutrition* **12**: 205-214, 1936.

THE ASPECT OF THE BANDS OF SCHREGER IN THE HORIZONTAL SECTIONS OF THE ENAMEL

JORGE ERAUSQUIN

Catedra de Histologia y Embriologia, Escuela de Odontologia, Universidad de Buenos Aires, Argentina

I. INTRODUCTORY

THE bands of Schreger are a series of bands visible by reflected light in the zones corresponding to the diazones described in the enamel by Preiswerk (1909). There are some recent observations which support the idea that density of calcification may be involved in their formation. Hollander, Bodecker, Applebaum, and Saper (1935), using soft x-rays, found differences in the radiopacity of the bands of Schreger. Staz (1946) suggested that they may indicate stages in the calcification process, considering the concentric disposition of the bands and its correspondence to the calcification process of the enamel (Diamond and Weinmann, 1940; Weinmann, Wessinger, and Reed, 1942; Orban, Sicher and Weinmann, 1943; and others).

However, it seems quite evident that the main factor in the formation of the bands of Schreger is the manner in which the section shows the enamel rods, the diazones, and parazones. On the other hand, there is no satisfactory reason to explain why the bands of Schreger show regularity and approximately horizontal disposition and why they are usually visible in the lateral plates and in the inner two thirds of the enamel. Moreover, the bands of Schreger do not appear in horizontal sections. However, we cannot accept the statement that the deviation of the enamel rods which determines the diazones and parazones cannot be observed in horizontal sections. What is the aspect of those diazones and parazones, and consequently, of the bands of Schreger in the horizontal sections?

II. GENERAL ASPECT

The bands of Schreger appear as fusiform stripes, perpendicular to the dentino-enamel junction. They can be seen to have a slight curvature with its concavity toward the incisal or cuspal portion. As they are perpendicular to the dentino-enamel junction, they incline progressively toward the incisal portion. This obliquity may be 35 or 40 degrees in some cases and, in a general way, resembles the concentric arrangement described by Staz (1946).

The bands of Schreger do not occupy the whole thickness of the enamel. Generally, they start at or near the dentino-enamel junction and from there they run through the inner two thirds, ending progressively in the outer third of

Received for publication October 15, 1947, revised by author April 14, 1948.

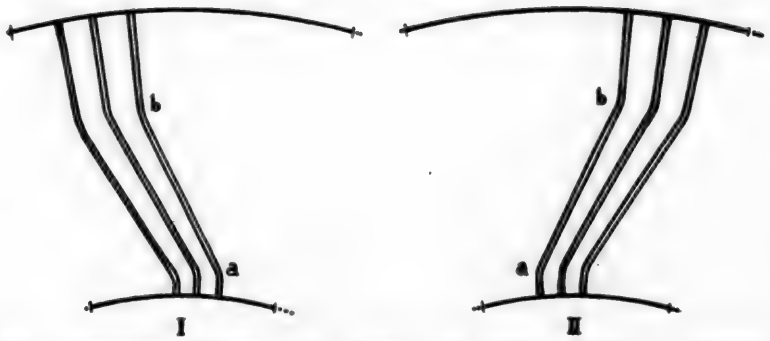


Fig. 1.—Horizontal deviation of the prisms in the rings of enamel. *I.* Deviation to the left. *II.* Deviation to the right. *a.* First curve, close to the amelodentinal junction. *b.* Second curve, close to the outer third of the enamel.

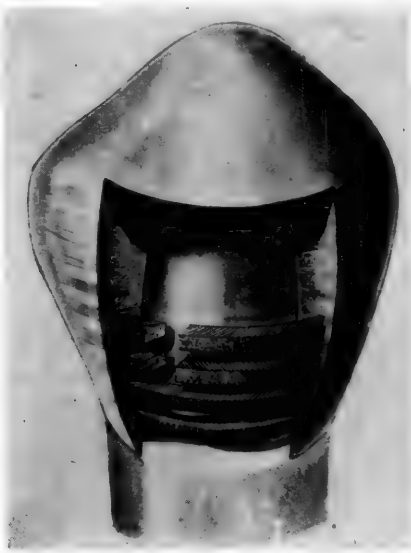


Fig. 2.

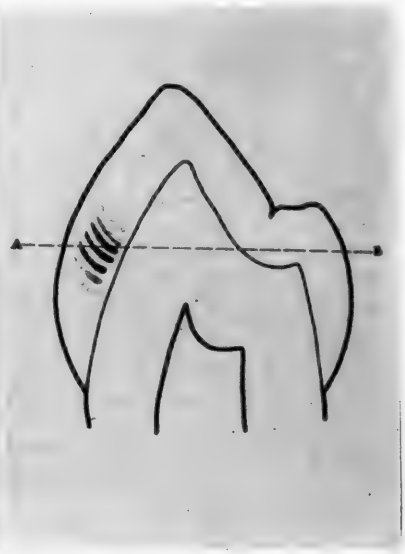


Fig. 3.

Fig. 2.—Schematic tridimensional representation of the alternate disposition of the enamel rings.

Fig. 3.—Scheme to show that a horizontal section may cut through two or three consecutive bands of Schreger.

the enamel. It will be shown later that this localization corresponds to the different orientation shown by the enamel rods in the inner two thirds compared with the outer third of the enamel.

It has just been noted that these curvatures of the prisms are disposed in horizontal layers. In each one of these layers the prisms are perpendicular to the dentino-enamel junction and a short distance later, they change their direction to the right or to the left, always maintaining the horizontal plane. Subsequent to this first curve, another one follows in the opposite direction, and in this way the prisms resume a radial direction which is maintained to the

Fig. 4.—Longitudinal section of enamel. Aspect of the bands of Schreger. The rings with the prisms deviating in one direction, appear like "diazonias." The rings with the prisms deviating in the other direction, appear like "parazonias."

periphery of the enamel. The same arrangement is repeated in the next layer but in opposite directions. For instance, if in the first one, the initial curve were to the right, in the next second one it will be the left, and vice versa. This prism deviation will continue in the same way in the next layers, forming horizontal layers in which the deviations appear alternately to the right and to the left.

B.

A.

Fig. 5.—Horizontal section of enamel.
A. The rings of enamel appear concentrically with their alternate direction to the right and to the left.
B. Semischematic representation of *A*.

III. STRUCTURE

The direction of the rods of the human enamel is not strictly radial. The rods show a frankly intricate disposition in the deep layers of the cuspal zones, the so-called gnarled enamel. However, in the lateral plates, the rods have a characteristic, nearly regular, double deviation that can be seen in horizontal layers. These deviations appear only in the deep layers of the enamel. The outer half of the enamel, or at least the outer third, is made up of straight and parallel rods. The structure is similar to that of the rods in the cuspal areas. Their disposition is very intricate in the deep layers of the cusps (gnarled enamel). In the lateral plates they show, in horizontal layers, a characteristic, double, quite even orientation. These deviations only appear in the deep zones of the enamel. Half, or at least the outer third, of the enamel is formed by straight and parallel prisms.

The double curvature decreases in those prisms nearest to the upper and lower planes in each ring till it disappears completely and is reversed in the central zone of the following ring. This is the reason that a longitudinal section cuts the prisms of the central zone of each ring in a very marked obliquity where the edges of the two contiguous rings constitute a transitional stripe among prisms orientated radially. The central zone of these rings corresponds to the diazones of Preiswerk, and the borderline edges of the two contiguous rings, without any demarcation between them, represents the parazones.

The planes in which these deviations are produced are represented horizontally. Especially in the vicinities of the cusps, these planes have a variable inclination in the axiocervical direction. Keeping this inclination all along the circumference in one slice, the result will be a conic surface or a flat infundibular one.

This obliquity of the rings of enamel is shown in the horizontal sections. As a matter of fact, in a variable degree, depending probably on crown shape and on the level the crown was cut, in a horizontal section simultaneously two, three, or four rings are seen as a consequence of its obliquity; they appear in concentric circles in the deepest zones of the enamel. Under low magnification it is seen that the concentric bands show the prisms deviated alternately to the right and to the left. In this way, there is evidence that they correspond to consecutive rings. The outer third of the enamel and a thin internal layer are shown radially in the horizontal sections.

SUMMARY

The bands of Schreger correspond to a horizontal double curvature of the prisms in the inner two thirds of the enamel in the lateral plates. This double curvature involves a complete ring of enamel. In different rings, the deviation is to the left or to the right, alternately.

The enamel rings are not completely flat but rather are slightly conic. For that reason a horizontal section cut through two or three of these rings, with the alternate deviation to the right and to the left, shows concentric rings.

REFERENCES

1. Diamond, M., and Weinman, J. P.: The Enamel of the Human Teeth, New York, Columbia University Press, 1940.
2. Hollander, F., Bodecker, C. F., Applebaum, E., and Saper, E.: A Study of the Band of Schreger by Histological and Grenz-Ray Methods, *N. Y. S. Den. Soc. Tr.* 126, 1934, and *D. Cosmos* **77**: 12, 1935.
3. Orban, B., Sicher, H., and Weinman, J. P.: Amelogenesis, *J. Am. Coll. Dentists* **10**: 13, 1943.
4. Preiswerk, Gustav: Atlas and Text-Book of Dentistry Including Diseases of the Mouth, Philadelphia and London, W. B. Saunders Company, 1909.
5. Staz, J.: Bands of Schreger, *J. D. Res.* **25**: 373-380, 1946.
6. Weinmann, J., Wessinger, G. D., and Reed, G.: Correlation of Chemical and Histological Investigations on Developing Enamel, *J. D. Res.* **21**: 171, 1942.